An Integrative Study of Patterns and Processes Driving Sexual Dimorphism and Life-History Evolution in Diptera and Other Insects

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Summary

This integrative dissertation explores the ultimate (evolutionary) as well as proximate (i.e. mechanistic developmental) drivers of life-history traits in insects. The focus lies on the evolution of body size, sexual size dimorphism (SSD) and sex-specific body size plasticity, which are studied on different levels of biological organization. The following six chapters integrate experimental and quantitative genetic studies with comparative approaches and aim at broadening our current knowledge on how the astonishing phenotypic variation observed across the tree of life came about and how it is maintained.

Chapter 1 explores global patterns of body size, sexual size dimorphism, relative wing size and geographic range size among 151 species of fruit flies (Diptera: Drosophilidae). In vertebrates, these traits accord fairly predictably with prominent ecogeographic "rules" (Bergmann's, Rensch's, Allen's, Rapoport's rules). However, the predictive power of these rules in invertebrates — and insects in particular — is very poor, at least in part due to lack of a mechanistic understanding of the drivers of such variation. As these traits are to some extent evolutionarily or ecologically interdependent, possible confounding effects between macroecological patterns are expected and might explain some of the apparent idiosyncrasy. Such interrelations are rarely considered. Here, I test the predictions of Bergmann, Rensch, Allen and Rapoport for a large number of drosophilids across the globe to investigate potential confounding effects between patterns. Although there is limited evidence for any confounding effects, I nevertheless demonstrate the usefulness of studying several macroecological patterns simultaneously, as it allows for a deeper, mechanistic understanding of ecogeographic variation.

In **chapter 2**, I assess quantitative genetic latitudinal differentiation in life history traits in the widespread sepsid fly *Sepsis fulgens* (Diptera: Sepsidae) across 13 populations spanning 20 degrees latitude from southern Italy to Estonia. Despite very short generation times, I found a converse Bergmann cline (smaller size at higher latitudes). As development time did not change with latitude (flat cline), integral growth rate thus likely declines towards the pole. At the same time, early fecundity, but not egg size, increased with latitude. Rather than being mediated by seasonal time constraints, the body size reduction in the northernmost flies from Estonia could suggest that these are marginal, edge populations, as when omitting them the body size cline became flat as well. Most of the other sepsid species investigated to date also show flat body size clines, a pattern that strikingly differs from *Drosophila.* I conclude that *S. fulgens* life history traits appear to be shaped by similar environmental pressures and selective mechanisms across Europe, be they adaptive or not. This reiterates the suggestion that body size clines can result as a secondary consequence of selection pressures shaping an entire life history syndrome, rendering them inconsistent and unpredictable in general.

Chapter 3 focusses on the evolution of sexual size dimorphism (SSD) and sex-specific body size plasticity. In insects, females are usually the larger and more plastic sex. However, because females are larger than males in most species, it is difficult to assess whether their greater plasticity is driven by selection on size or represents an effect of the female reproductive role *per se*. I here estimate sex-specific body size plasticity of populations and species that vary in the direction and extent of SSD, and show that males are typically more plastic than females if they are the larger sex. Hence, my findings indicate that primarily selection on size, rather than the reproductive role *per se*, drives the evolution of sex-specific body size plasticity. However, sepsid flies, and possibly Diptera in general, show a clear sexual asymmetry with greater male than female plasticity related to SSD, likely driven by strong sexual selection on males. Although further research controlling for phylogenetic and ecological confounding effects is needed, the patterns are congruent with theory suggesting that condition dependence plays a pivotal role in the evolution of sexual size dimorphism.

In **chapter 4**, I investigate the potential link between the extent of sexual dimorphism and sex-specific condition dependence among traits and species. Sexual selection can displace traits acting as ornaments or armaments from their viability optimum in one sex, ultimately giving rise to sexual dimorphism. The degree of dimorphism should hence not only mirror the strength of sexual selection, but also the net viability costs and benefits of trait maintenance at equilibrium. The ability of organisms to bear exaggerated traits will depend on their condition. More sexually dimorphic traits should therefore also exhibit greater sex differences in condition dependence. While this has been shown to

apply among traits within species, condition dependence and sexual dimorphism are also expected to correlate across the phylogeny. I investigated and quantified this prediction within and across 11 (sub)species of black scavenger flies that vary in their mating system. When estimating sex-specific condition dependence for seven sexual and non-sexual traits that vary in their sexual dimorphism, we not only found a positive relationship between the sex difference in allometric slopes (as our measure of condition dependence) and relative trait exaggeration among traits within species, but also across species for those traits expected to be under sexual selection in males. I additionally show species with more pronounced male aggression to have relatively larger and more condition-dependent male fore and mid legs. My comparative study suggests a common genetic/developmental basis of sexual dimorphism and sex-specific plasticity that apparently evolves across the phylogeny, and that the evolution of trait size consistently alters scaling relationships and thus contributes to the allometric variation of sexual armaments or ornaments in animals.

In **chapter 5**, I investigate the physiological basis of adaptive size variation in the yellow dung fly *Scathophaga stercoraria*, which shows pronounced male-biased sexual size dimorphism and strong body size plasticity. I estimate variation of a major physiological threshold, the critical weight, which is the mass at which a larva initiates pupariation. Critical weight is associated with sexual size dimorphism and sex-specific plasticity, and is thus a likely target of selection on adult size. Detailed larval growth trajectories derived from individuals raised at two food and temperature treatments further reveal that sex-specific size plasticity is mediated by faster initial growth of males that later becomes reduced by greater male weight loss during the wandering stage. Hence, I illustrate the importance of detailed assessments of ontogenetic growth trajectories for the understanding of adaptive size variation and discuss the mechanistic basis of size determination in shaping sex-specific phenotypic plasticity.

Chapter 6 is devoted to the effect temperature on the evolution of insect wings. Given its profound effect on biological systems, temperature is often held responsible for eliciting phenotypic plasticity as well as quantitative genetic differentiation. If genetic and plastic responses to temperature are adaptive, they should be related in magnitude and form, a pattern that should evolve repeatedly in different lineages. I quantified this putative relationship between quantitative genetic latitudinal variation in wing loading and wing shape and their thermal plasticity in two closely related sepsid flies with contrasting sexual size dimorphism. Common garden rearing revealed decreasing wing loading with latitude independently in both species, likely driven by selection for increased dispersal capacity in the cold. Thermal plasticity for wing loading was however non-linear, suggesting that the relationship between plasticity and genetic differentiation is more complex. Although both species showed similar patterns of wing shape allometry, sexual dimorphism and thermal plasticity, latitudinal differentiation only mirrored thermal plasticity in one but not the other species. Arguing that such discrepancies may be driven by variation in gene flow and demography, these results reiterate the notion that genetic wing shape differentiation may be complex and idiosyncratic even among ecologically similar closely related species.

In summary, by integrating studies on different insect systems at multiple levels of biological organization (from single genotypes to population differentiation within species to global interspecific variation), this dissertation provided insights into the evolution of life histories and hence contributes to the understanding of diversity and disparity in the broadest sense.

Zusammenfassung

Diese integrative Dissertation untersucht die ultimativen (evolutionären) sowie die proximaten (mechanistischen) Ursachen von lebensgeschichtlichen Merkmalen bei Insekten. In Vordergrund steht die Evolution der Körpergrösse, des sexuellen Körpergrössendimorphismus und der geschlechtsspezifischen Körpergrössenplastizität, welche auf verschiedenen biologischen Ebenen untersucht werden. Die folgenden sechs Kapitel integrieren experimentelle und quantitativ-genetische Studien mit vergleichenden Ansätzen und zielen darauf ab, unser gegenwärtiges Wissen darüber zu erweitern, wie die erstaunliche phänotypische Variation in der Tierwelt zustande kam und wie sie aufrechterhalten wird.

Kapitel 1 untersucht globale Muster der Körpergrösse, des sexuellen Körpergrössendimorphismus, der relativen Flügelgrösse und der Grösse des geographischen Verbreitungsgebiets von 151 Fruchtfliegenarten (Diptera: Drosophilidae). Bei Wirbeltieren folgen diese Merkmale oft prominenten ökogeografischen "Regeln" (Regeln von Bergmann, Rensch, Allen, Rapoport). Jedoch ist die Vorhersagekraft dieser Regeln bei wirbellosen Tieren — und insbesondere bei Insekten — sehr gering. Dies zumindest teilweise weil ein mechanistisches Verständnis deren Ursachen fehlt. Da all diese Merkmale in gewissem Masse evolutionär oder ökologisch voneinander abhängig sind, sind Zusammenhänge zwischen makroökologischen Mustern zu erwarten. Ob solche Wechselbeziehungen generelle Muster beeinflussen wird allerdings selten in Betracht gezogen. Ich untersuche die Vorhersagen von Bergmann, Rensch, Allen und Rapoport auf globaler Ebene, um mögliche Wechselwirkungen zwischen den verschiedenen Mustern zu untersuchen. Obwohl es nur wenige Anhaltspunkte für direkte Zusammenhänge gibt, demonstriere ich dennoch die Nützlichkeit, mehrere makroökologische Muster gleichzeitig zu untersuchen, da dies ein tieferes, mechanistisches Verständnis der ökogeographischen Variation ermöglicht.

In **Kapitel 2** untersuche ich die quantitativ-genetische Populationsdifferenzierung verschiedener Merkmale bei der weit verbreiteten Schwingfliege *Sepsis fulgens* (Diptera: Sepsidae). Trotz ihrer sehr kurzen Generationszeiten variiert deren Körpergrösse nur schwach mit dem Breitengrad (etwas kleiner in höheren Breiten). Gleichzeitig nimmt der frühe Reproduktionserfolg (Eianzahl), nicht aber die Eigrösse, mit der Breite leicht zu. Die meisten der bisher untersuchten Sepsidenarten weisen ebenfalls flache Klinen auf, ein Muster, das sich von *Drosophila*-Arten deutlich unterscheidet. Ich schliesse daraus, dass die untersuchten Merkmale von *S. fulgens* über ganz Europa hinweg von ähnlichen Umwelteinflüssen und selektiven Mechanismen geprägt sind. Dies weist erneut darauf hin, dass Körpergrössensklinen als sekundäre Folge von Selektionsdrücken auftreten können, die ein gesamtes Lebensverlaufssyndrom formen, und somit Klinen im Allgemeinen uneinheitlich und unberechenbar machen.

Kapitel 3 befasst sich mit der Evolution des sexuellen Körpergrössendimorphismus (sKGD) und der geschlechtsspezifischen Plastizität der Körpergröße. Bei Insekten sind Weibchen normalerweise das grössere und plastischere Geschlecht. Da Weibchen jedoch bei den meisten Arten grösser sind als Männchen, ist es schwierig zu beurteilen, ob ihre stärkere Plastizität durch Grössenselektion an sich oder generell durch ihre Geschlechterrolle bei der Fortpflanzung bestimmt wird. Um dies zu testen, untersuche ich die geschlechtsspezifische Körpergrössenplastizität von Populationen und Arten, die sich in der Richtung und dem Ausmass des sKGD unterscheiden. Wie sich herausstellt, sind Männchen typischerweise plastischer wenn sie auch grösser sind als die Weibchen. Daher deuten meine Ergebnisse darauf hin, dass primär die gerichtete Körpergrössenselektion und nicht die reproduktive Rolle als solche die Evolution der geschlechtsspezifischen Körpergrössenplastizität vorantreibt. Obwohl weitere Untersuchungen zur Kontrolle phylogenetischer und ökologischer Effekte erforderlich sind, stimmen die Muster mit der Vermutung überein, dass die geschlechtsspezifische Konditionsabhängigkeit eine entscheidende Rolle in der Evolution des sKGD spielt.

In **Kapitel 4** untersuche ich einen putativen Zusammenhang zwischen Geschlechtsdimorphismen und der geschlechtsspezifischen Konditionsabhängigkeit bei verschiedenen Merkmalen und Arten. Sexuelle Selektion kann Merkmale, die als Ornamente oder Waffen fungieren, von ihrem Selektionsoptimum unter natürlicher Selektion verdrängen. Da dies häufig nur bei einem Geschlecht passiert, führt dies zur Evolution von sexuellem Dimorphismen. Der Grad des Dimorphismus sollte daher nicht nur die Stärke der gerichteten sexuellen Selektion, sondern auch die Vor-und Nachteile der Merk-

malsausprägung im Selektionsgleichgewicht widerspiegeln. Die Fähigkeit von Organismen, stark ausgeprägte Merkmale auszubilden, hängt allerdings von ihrer Kondition (im Sinne der Leistungsfähigkeit) ab. Geschlechtsspezifische Unterschiede in der merkmalsspezifischen Konditionsabhängigkeit sollten daher mit der Ausprägung des Sexualdimorphismus zunehmen. Da dies bereits bei Merkmalen innerhalb Arten nachgewiesen wurde, sollten die Konditionsabhängigkeit und der Geschlechtsdimorphismus auch zwischen Arten korrelieren. Diese Vorhersage habe ich bei 11 Schwingfliegen(unter)arten untersucht, die sich stark in ihrem Paarungssystem unterscheiden. Die Geschlechtsunterschiede in den allometrischen Steigungen (d.h. der Zusammenhang der Merkmals- mit der Körpergrösse) – mein Mass für Konditionsabhängigkeit – nehmen in der Tat mit der Stärke der sexuellen Dimorphismen zu. Dieses Muster wurde nicht nur für Merkmale innerhalb Arten, sondern auch über Arten hinweg gefunden. Geschlechtsdimorphismen sowie die geschlechtsspezifische Plastizität weisen daher eine gemeinsame genetische bzw. entwicklungsbiologische Grundlage auf, und sind so konzeptionell nur schwierig zu unterscheiden.

In **Kapitel 5** untersuche ich die physiologische Basis der adaptiven Grössenvariation bei der gelben Dungfliege *Scathophaga stercoraria*. Bei dieser Art sind Männchen um einiges grösser als Weibchen und zeigen eine ausgeprägte Körpergrössenplastizität. Um die Wachstumsunterschiede zwischen den Geschlechtern und Umwelten besser zu verstehen, wird die genetische und umweltspezifische Variation des sog. „kritischen Gewichts" untersucht. Dieser physiologische Schwellenwert stellt dasjenige Gewicht dar, bei dem die Larve ihre Verpuppung einleitet. Das kritische Gewicht korreliert mit dem sexuellen Körpergrössendimorphismus sowie der geschlechtsspezifischen Plastizität und wird daher wahrscheinlich indirekt durch Selektion im Adultstadium beeinflusst. Des Weiteren zeigen detaillierte Wachstumskurven, die bei zwei Nahrungs- und Temperaturmanipulationen erhoben wurden, dass die geschlechtsspezifische Grössenplastizität durch ein schnelleres anfängliches Wachstum der Männchen zustande kommt. Dies illustriert die Bedeutung der Erhebung detaillierter Wachstumskurven für das Verständnis adaptiver Grössenvariation. Die mechanistische Basis der Ermittlung der eigenen Grösse einer Larve bei der Gestaltung geschlechtsspezifischer phänotypischer Plastizität wird in diesem Zusammenhang diskutiert.

Kapitel 6 ist der Evolution und Temperaturabhängigkeit der Morphologie von Insektenflügeln gewidmet. Aufgrund ihrer tiefgreifenden Wirkung auf biologische Systeme wird die Temperatur häufig für phänotypische Plastizität sowie für die quantitative genetische Differenzierung verantwortlich gemacht. Falls genetische wie plastische Reaktionen auf die Temperatur in der Tat adaptiv sind, sollten sich deren Effekte in Form und Stärke ähneln, ein Muster, das sich bei verschiedenen Arten wiederholt zeigen sollte. Diese mutmassliche Beziehung wird an Hand der klinalen Variation und Temperaturplastizität der relativen (d.h. grössenkorrigierten) Flügelgrösse und -form zweier eng verwandter Sepsiden untersucht. Beide Arten zeigen eine Zunahme der relativen Flügelgrösse mit dem Breitengrad. Dies ist ein genetisches, konvergent evolviertes Muster, welches wahrscheinlich durch verstärkte Selektion auf die Flugkapazität in kalten Klimaten zustande kommt. Gleichzeitig zeigt die Temperaturplastizität der relativen Flügelgrösse ein nicht lineares Muster, was auf eine komplexe Beziehung zwischen Plastizität und genetischer Differenzierung hindeutet. Obwohl beide Arten ähnliche Muster der Flügelform-Allometrie, des Sexualdimorphismus und der Temperaturplastizität aufwiesen, war die genetische Differenzierung nur bei einer der Arten mit der Temperaturplastizität korreliert. Diese Artunterschiede sind möglicherweise durch Variation in der Stärke des Genflusses oder der Demografie bedingt und bestätigen die Ansicht, dass die genetische Flügelformdifferenzierung sogar bei ökologisch ähnlichen, eng verwandten Arten schwer vorhersagbar sein kann.

Durch die Integration von experimentellen, quantitativ-genetischen und vergleichenden Studien verschiedener Insektengruppen auf diversen biologischen Ebenen (von einzelnen Genotypen über Populationsunterschiede innerhalb Arten bis hin zu globalen interspezifischer Variation) liefert diese Dissertation Einblicke in die Evolution von Lebenszyklen und trägt somit zum Verständnis des Ursprungs und der Aufrechterhaltung der Diversität und Disparität im Tierreich bei.

General Introduction

Although of longstanding interest, understanding the processes that promote and maintain the vast diversity and disparity across the tree of life remains one of the prime aims of evolutionary biology. Body size is a highly variable phenotypic trait that most closely relates to Darwinian fitness globally across the animal kingdom (Stearns and Koella 1986) and has therefore been subject to considerable scrutiny. Size varies tremendously within organisms during ontogeny, differs between individuals of the same population due to environmental or genetic variation, and shows drastic variation between species. Large individuals generally have more offspring, acquire more mates, live longer, and are more competitive in acquiring resources. However, large body size typically also imposes costs in terms of prolonged development, increased parasitism or predation, late reproduction, and increased demand for resources (Blanckenhorn 2000, 2005, 2007, Honek 1993; Preziosi and Fairbairn 2000; Peters 1986; Reiss 1991). Hence, differences in size or growth between groups of individuals (be it genotypes, populations or species) are expected to reflect the net outcome of opposing selection pressures to a large extent.

Given the tight relationship of body size with various fitness components, it is necessary to study and disentangle the effects of several forms of selection simultaneously (Fairbairn and Preziosi 1994, Preziosi and Fairbairn 2000). As possible selective drivers are numerous, ranging from propagule survival to predation pressure and copulation success, it is often difficult to pinpoint the actual causes of size variation. Moreover, genetically, body size is a particularly complex trait influenced by a plethora of loci affecting a multitude of molecular pathways linked to size determination and growth (Mirth et al. 2008, Mirth and Riddiford 2007, Nijhout et al. 2014, Shingleton 2011, Nijhout and Davidowitz 2009). Lastly, body size is evolutionary and ecologically integrated with many other critical life-history traits such as growth, development, reproduction, survival, etc. in complex life history syndromes (Nylin and Gotthard 1998, Roff 2002). It has therefore proven difficult to link the evolutionary drivers of body size variation with their proximate (mechanistic) determinants. A complete understanding of why and how body size evolves (or not) hence requires the study of a whole array of related life-history traits.

Scope of the dissertation

This dissertation deals with the ultimate (evolutionary) as well as the proximate (mechanistic) drivers of body size and life history variation in the broadest sense, focussing mostly on insects, dipterans in particular. It aims at integrating different subdisciplines, ranging from physiology to macroecology, to acquire a more complete understanding of body size evolution and life histories in general. As argued repeatedly in the following chapters, the findings reported here are thus not necessarily limited to insects (or arthropods, for that matter).

Chapters 1, 2, 3 & 5 have been already peer-reviewed and published in international journals. Chapter 4 is currently in (second minor and hopefully final) revision at The American Naturalist; and chapter 6 is a manuscript prepared for submission to Journal of Evolutionary Biology.

In the following introductory sections, I briefly outline some of the key concepts and frameworks that have been used to understand body size evolution, and which. I have tried to integrate throughout my dissertation chapters.

Body size in Holometabola

In Holometabola, i.e. insects with complete metamorphosis, the evolution of body size appears particularly intriguing. First, as is typical for all insects, Holometabola only grow during their larval stages, and the size of the adult is predetermined well before the onset of metamorphosis (but see e.g. Molleman et al. 2011). This implies that individuals make their decision on when to stop growing at a stage when the ecological selection pressures that will affect the adult stage later in life may not be well predictable. Second, larvae, pupae and adults often have drastically different life styles. Immature stages frequently feed on different substrates and dwell in habitats entirely different from those of the adult. While this limits competition between stages and enables these insects to inhabit and exploit a very broad range of habitats, ecological selection acting on body size and other traits is very likely to vary strongly between life stages. If selection is an-

tagonistic, this can impose constraints on life-history evolution (Nylin and Gotthard 1998). Third, Holometabola comprise a majority of multicellular animal species on this planet, documenting an unprecedented disparity of life on earth (e.g. Stork et al. 2015). Hence, understanding how this group produced and maintained such vast variation in size and other traits is of general interest.

Ecogeographic size variation

During the 19th century, the study of interspecific body size variation across time and space caught the attention of early physiologists who eagerly and successfully demonstrated consistent size variation in relation to climate and other variables of interest. This is because environmental gradients often impose strong, predictable patterns of selection that can consequently fuel (local) adaptation (Endler 1977). Among others, work by Cope (1885; species become larger with geological time), Allen (1877; appendage size decreases with latitude), Bergmann (1847; body size increases with latitude), or Rensch (1950; sexual size dimorphism increases with size) have most prominently contributed to our understanding of ecogeographical patterns across species. However, while demonstrated to apply repeatedly, particularly in vertebrates, these so-called macroecological "rules" are rather poorly supported in invertebrates. Especially in insects patterns within and across species are so idiosyncratic that some authors refuse to attribute any predictive power to some of these rules (e.g. Shelomi 2012). As I argue later, this may not merely be due to our currently limited understanding of the mechanisms in action, but also due to the lack of appreciation of interrelated and possibly counteracting patterns of different life-history traits.

Sexual dimorphism

Sexual dimorphism, the divergence of morphological, behavioral and physiological traits between the sexes, is predicted to reflect the adaptive divergence between the sexes driven by variation in the optimal character states for each sex, thus representing an epiphenomenon (Bateman 1948; Hedrick & Temeles 1989). Since the costs and benefits of mating virtually always differ between the sexes, sexual dimorphisms are ubiquitous in all sorts of traits among dioecious species (Abouheif & Fairbairn 1997; Bateman 1948; Darwin 1872). Due to its strong correlation with physiology and fitness, body size is especially subject to sexual dimorphism. Sexual size dimorphism (SSD) arises whenever the net effects of natural and sexual selection on body size differ between the sexes, as proposed by the differential equilibrium model of the evolution of SSD (Andersson 1994; Blanckenhorn 2000; Preziosi & Fairbairn 2000; Price 1984). In most mammals and birds, sexual selection on male body size, be it caused by male-male competition or female choice, is *relatively* stronger than fecundity selection on female body size, leading to male-biased SSD (Abouheif & Fairbairn 1997). In invertebrates, such as arthropods, in contrast, fecundity selection on female size is generally stronger than sexual selection on male size, thus leading to female-biased SSD (Abouheif & Fairbairn 1997; Blanckenhorn et al. 2007; Hirst & Kiorboe 2014). Nevertheless, there are numerus examples throughout the Insecta in which male-biased sexual size dimorphism has evolved, often in association with increased male-male competition.

SSD is often regarded as a rather static, genetically determined trait. While this is certainly the case, it has long been appreciated in quantitative genetics that the total phenotypic variance of quantitative traits (V_P) is not only composed of genetic variance (V_G), but also dependent on environmental variation (V_E) and the way different genotypes respond to environmental fluctuations (so-called genotype-environment interactions: V_{GxE} (Lynch and Walsh 1998); although there are further sources of variation such as maternal and dominance effects). This clearly also applies to body size and possibly also to SSD. Sex-specific body size (i.e. developmental) plasticity in response to different environments is therefore expected, but its relationship to the extent and direction of SSD has remained underappreciated (Stillwell et al. 2010).

Variation in growth and size determination

Whereas the selective forces shaping SSD are quite well understood, the developmental mechanisms and underlying genetic architecture remain poorly investigated despite its importance for understanding the evolutionary process (Badyaev 2002; Stillwell et al. 2010). Adult size is necessarily a function of the speed and duration of growth during the juvenile stages. Total development time and growth rate are therefore often seen as the primary targets of selection. However, there are at least two issues related to the use of estimates for development time and growth rates. First, insects neither grow continuously, nor in a linear or exponential fashion (Tammaru & Esperk 2007, Tammaru et al. 2010). It is thus methodologically difficult to derive precise estimates of growth in the first place. Consequently researchers often use simple, linear measures of

growth such as egg-to-adult development time and integral growth rates (ratio between adult size and total developmental duration). Such measures can be misleading as they are only crude approximations of a much more complex phenotype (but see Davidowitz 2016). But admittedly, it is often impossible to come up with measurements other than simple ratios between adult size and total egg-to-adult development time, especially when having to rely on published records for meta analyses.

There is a second issue that might be of even greater concern: body size may only indirectly depend on the rate or duration of growth in the first place. At least as important are the mechanisms that terminate growth at a particular size or point in time (Nijhout 2003; Nijhout & Davidowitz 2009). Thus, when studying body size variation, be it environmentally plastic or genetically fixed, consideration of the complexity of larval growth, as well as mechanisms of size determination, is crucial for our understanding. Apart from a few model species, how size is sensed and determined during growth, however, is not well studied. This is surprising and unfortunate due to its immediate implication for body size evolution.

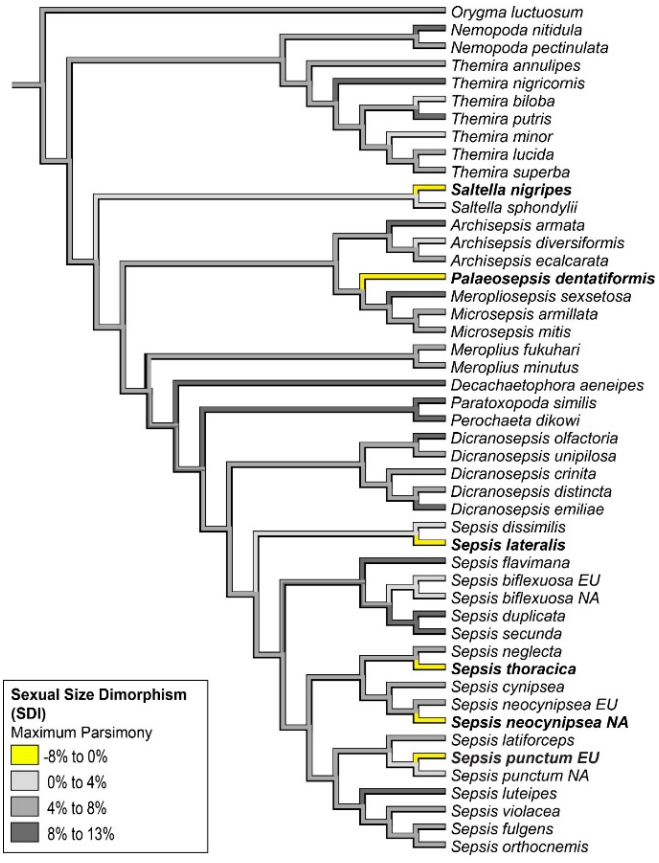

Figure 1: Phylogenetic reconstruction of sexual size dimorphism (SDI) using Wagner's linear parsimony reveals that female-biased sexual size dimorphism is predominant and ancestral in sepsids; however, male-biased dimorphism has evolved at least six times independently. Negative values indicate male-biased SDI (yellow), whereas positive values denote a graded extent of female-biased SDI (gray scale). (figure from Rohner et al. 2016)

Body size evolution in sepsid flies (Diptera: Sepsidae)

Although I have studied several different insect systems, most of my work has focused on black scavenger flies. Sepsids represent a family-ranked clade of acalyptrate dipterans strongly associated with the decomposition of organic matter (Pont & Meier 2002). By now, this group has been widely studied in evolutionary, behavioral, ecological, ecotoxicological, phylogenetic, and developmental research contexts (eg. Berger et al. 2013; Blanckenhorn et al. 2013; Bowsher & Nijhout 2007, 2009; Eberhard 2001a-c, 2003; Puniamoorthy 2014, Puniamoorthy et al. 2009, 2012a,b; Rohner et al. 2015, 2016). This is mostly due to (i) our ability to cultivate many species in the laboratory without much effort in standardized ways, both as populations and as genetic iso-female lines; (ii) the fast development and easy experimental handling; (iii) the large variation in life history, morphology and mating systems even among closely related species; and (iv) their role as decomposers in agricultural grasslands worldwide. Further, (v) the phylogenetic relationships among species have been resolved (Meier 1995a; Meier 1995b; Meier 1996; Zhao et al. 2013), such that inferences are robust and allowing for comparative work.

Sepsids are particularly well suited to study the evolution of body size, sexual size dimorphism and sex-specific plasticity, as recent findings document directional variation in SSD within species, which is exceptionally rare in the animal kingdom. In her unpublished dissertation, Katja Schulz (1999) first noted a cross-continental reversal of SSD in *Sepsis punctum,* which was then formally documented by Puniamoorthy et al. (2012a,b): whereas males are the larger sex in Europe, North American populations express female-biased SSD. A similar, but reversed pattern was revealed by my own master's work on *Sepsis neocynipsea* (Rohner et al. 2016). In this species, females are the larger sex in Europe, whereas in North America SSD is male-biased. When testing the differential equilibrium model of SSD we found that sexual selection alone can account for the directional reversal of SSD in both species, whereas fecundity selection is invariant across the species' ranges. These reversals in SSD are closely linked to shifts in the mating system, behavior and the morphology of primary and secondary sexual traits (Dmitriew & Blanckenhorn 2014b;

Puniamoorthy et al. 2012b), and hence open a window into body size evolution in general.

In addition to these replicated intraspecific directional reversals of SSD, male-biased SSD has evolved repeatedly elsewhere in the sepsid phylogeny (e.g. in *Sepsis lateralis, Sepsis thoracica, Palaeosepsis dentatiformis, Saltella nigripes*: Rohner et al. 2016, fig. 1). Due to the convergent evolution of SSD reversals, this group of closely related, widespread species is ideal for studying the proximate mechanisms leading to sexually dimorphic body size, and in particular for testing whether the convergent evolution of male-biased SSD is mediated by the same shifts in the ontogenetic control of dimorphism.

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ECOGRAPHY

Research

Interrelations of global macroecological patterns in wing and thorax size, sexual size dimorphism, and range size of the Drosophilidae

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Support for macroecological rules in insects is mixed, with potential confounding interrelations between patterns rarely studied. We here investigate global patterns in body and wing size, sexual size dimorphism and range size in common fruit flies (Diptera: Drosophilidae) and explore potential interrelations and the predictive power of Allen's, Bergmann's, Rensch's and Rapoport's rules. We found that thorax length ($r^2 = 0.05$) and wing size ($r^2 = 0.09$) increased with latitude, supporting Bergmann's rule. Contrary to patterns often found in endothermic vertebrates, relative wing size increased towards the poles ($r^2 = 0.12$), a pattern against Allen's rule, which we attribute to selection for increased flight capacity in the cold. Sexual size dimorphism decreased with size, evincing Rensch's rule across the family ($r^2 = 0.14$). Yet, this pattern was largely driven by the *virilis–repleta* radiation. Finally, range size did not correlate with latitude, although a positive relationship was present in a subset of the species investigated, providing no convincing evidence for Rapoport's rule. We further found little support for confounding interrelations between body size, wing loading and range size in this taxon. Nevertheless, we demonstrate that studying several traits simultaneously at minimum permits better interpretation in case of multiple, potentially conflicting trends or hypotheses concerning the macroecology of insects.

Keywords: Allen's rule, Bergmann's rule, clinal variation, Diptera, Drosophilidae, dispersal, Rapoport's rule, Rensch's rule, wing loading

Introduction

Convergent patterns of phenotypic variation across large-scale environmental gradients have long been recognized and have given rise to several macroecological 'rules' predicting such variation as putative adaptive responses to selection. These patterns are generally supported by empirical evidence and underlie theory. Until a more complete understanding of the selective mechanisms underlying the patterns is achieved, however, the predictive power of such rules must remain limited. This

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is particularly true for insects, in contrast to mammals and birds (Blanckenhorn and Demont 2004, Chown and Gaston 2010, Shelomi 2012).

As in all organisms, insect body size is inherently linked to physiology, metabolic rate, survival and reproductive success and is thus thought to evolve in predicted ways if subjected to similar selective drivers (Blanckenhorn 2000, Chown and Gaston 2010). Large size typically increases mating success in males and fecundity in females, but can entail heightened mortality risks and reproductive costs due to prolonged juvenile development (Blanckenhorn 2000). Amongst the macroecological rules proposed to account for body size variation, Bergmann's rule, signifying an increase in size with latitude, is well supported in homoeothermic vertebrates (Bergmann 1847, Meiri and Dayan 2003), but its absence and often its converse is prominent among invertebrates (Shelomi 2012). An increase in size with latitude has been attributed to temperature-dependent variation in growth and metabolic rates, a pattern congruent with the so-called temperature–size rule (Atkinson 1994, Atkinson and Sibly 1997) that generally predicts insects to grow bigger in the cold (Kingsolver and Huey 2008). However, a shortened active season with increasing latitude can also cause adaptive negative size clines through selection for fast development if development cannot be extended across one season, both within and between species (Chown et al. 1999, Blanckenhorn and Demont 2004, Zeuss et al. 2017). Small insects with rapid development are thus expected to follow Bergmann clines, whereas large insects may be limited in their development by season length at high latitudes, thus emerging smaller and showing converse Bergmann clines (Blanckenhorn and Demont 2004, Zeuss et al. 2017).

In contrast to body size, the relative size of appendages has been found to decrease with latitude in endothermic vertebrates (Nudds and Oswald 2007, Symonds et al. 2010). Termed Allen's rule, this pattern has received considerable attention and has been attributed to selection for a reduced surface-to-volume ratio to limit heat loss in the cold. Originally documented for warm-blooded animals (Allen 1877), qualitatively similar patterns have also been described in invertebrates (Alpatov 1929, Ray 1960). In insects, most appendages, including wings and antennae, are connected to the circulatory system (Chapman et al. 2013), and the constant flow of haemolymph through these appendages can contribute to thermoregulation, as in endotherms. Patterns equivalent to Allen's rule might thus be expected. Still, particularly for small insects, body temperature is unlikely to be strongly dependent on the relative surface area as an insect's body adjusts nearly instantly to the ambient temperature (Harrison and Roberts 2000), although many insect taxa are capable of considerable thermoregulation (social Hymenoptera in particular; Stabentheiner et al. 2010, Chapman et al. 2013). Consequently, small insects primarily regulate their body temperature by modifying their behavior (Clench 1966, Dillon et al. 2009), thus making best use of available microhabitats. The capacity to disperse, however, is greatly restricted at cool temperatures, which impedes take-off in winged insects (Dillon and Frazier 2006, Frazier et al. 2008). As increased wing size relative to body size facilitates take-off at cooler temperatures (Frazier et al. 2008), a latitudinal increase of relative wing size (a pattern counter to Allen's rule) can be predicted. Such patterns have indeed been observed in insects (e.g. clinal population differentiation in *D. melanogaster*: Azevedo et al. 1998), but the repeatability of such clines awaits further scrutiny. Whether the relative size of insect appendages increases, decreases or shows any consistent latitudinal pattern at all thus remains unclear.

Whereas both Bergmann's and Allen's rules describe spatial variation in body and appendage size irrespective of sex, the widely-studied Rensch's rule focuses on variation in sexual size dimorphism (SSD; Abouheif and Fairbairn 1997, Fairbairn 1997). Rensch (1950) documented that, among closely related species of many disparate taxonomic groups, SSD increases with body size in species in which males are the larger sex but decreases when females are larger than males. Unlike Bergmann's and Allen's rules, which capture size variation that likely results from natural selection, differential variation between the sexes has been attributed to a combination of sexual selection on males (e.g. via male–male contest competition) and fecundity selection on females (Fairbairn and Preziosi 1994, Fairbairn 1997, Székely et al. 2004). However, evidence for Rensch's rule is mixed, particularly in taxa with female-biased size dimorphism, such as most insects and spiders (for which SSD tends to increase with body size: Blanckenhorn et al. 2007b, Webb and Freckleton 2007, Stuart-Fox 2009), undermining its predictive power.

Finally, in addition to body size, populations or species have also been documented to vary in their range size. Specifically, Rapoport's rule (or 'effect': Stevens 1992) predicts that species occurring close to the equator will have more restricted ranges because they exhibit limited climatic tolerance as a result of adapting to a local environment with low climatic variability. Conversely, species occurring at higher latitudes (or altitudes), which feature ample climatic variability, are predicted to be better adapted to colonize and occupy more diverse habitats. Evidence for this rule is still controversial as it might be restricted to specific latitudes and certain regions and somewhat scale dependent (Rohde 1996, Ruggiero and Werenkraut 2007).

The four macroecological patterns described above are often studied in isolation from each other. Nevertheless, body size, wing morphology (which relates to dispersal capacity; Ray et al. 2016), SSD and range size are all likely to be ecologically and evolutionarily interrelated, if only because most adaptive explanations discussed above relate to climate. For example, although Rensch's rule has mostly been studied independently of environmental factors, latitudinal patterns of SSD have been observed (Blanckenhorn et al. 2006), and a relationship of Rensch's rule with the widely-observed temperature–size rule was postulated but not found after all (Hirst et al. 2015). Similarly, a large body of literature investigates relationships between range size and both dispersal capacity and body size (Gaston and Blackburn 1996, Malmqvist 2000, Lester et al. 2007, Rundle et al.

2007, Laube et al. 2013), thus raising the issue of whether clinal variation in size and dispersal might generally drive Rapoport's rule, possibly undermining its ecological relevance (Reed 2003). It is thus useful – if not imperative – to account for additional potentially confounding effects when investigating range-size variation (Laube et al. 2013).

Rigorous examination of macroecological patterns requires not only robust taxon sampling and coverage of a large geographic range, but also the ability to account for phylogenetic relationships among species. The Drosophilidae, a highly speciose and diverse family of fruit flies with a global distribution, should be highly suited to assess such patterns and their underlying mechanisms in insects. Many drosophilids are cosmopolitan generalists, whereas others are highly specialized and endemic to small geographic areas (Ashburner 1981). In this taxon, Bergmann's rule has received considerable attention at the intraspecific level along both latitudinal and altitudinal gradients (e.g. *D. melanogaster*: van't Land et al. 1999, Klepsatel et al. 2014, Fabian et al. 2015; *D. buzzatii*: Karan et al. 2000; *D. subobscura*: Gilchrist et al. 2001; *Zaprionus indianus*: Karan et al. 2000), but little information is available at the interspecific level. Similarly, Rensch's rule has been addressed in the *obscura* group (Huey et al. 2006), but the general pattern has not been investigated (but see Blanckenhorn et al. 2007a, b). The potential relationship of range size with body and appendage size also remains largely unexplored in this group.

We here assessed the ecogeographical patterns of thorax length and wing size, SSD, and range size in 151 drosophilid species from around the globe, including members of all three major *Drosophila* clades plus species of other genera belonging to this speciose family. Accounting for phylogenetic non-independence based on a reconstructed phylogeny, we investigated the geographical patterns described above and potential relationships between the four macroecological rules. Such relationships are expected under the prediction that they all relate (to some extent) to climate, but their covariation is poorly studied empirically. We thus aimed to better understand the causes and consequences of macroecological variation.

Material and methods

Phylogeny reconstruction

To reconstruct the drosophilid phylogeny, we obtained the sequences of six nuclear, three mitochondrial and three ribosomal genes from GenBank (see Supplementary material Appendix 1 Table A1 for accession numbers and sequence coverage). The gene coverage per species ranged between 1 and 12 (mean ± SD = 7.2 ± 2.9 loci/species), with a total sequence length of 6269.2 ± 3267.6 bp (range = 337–14 449 bp). The nuclear sequences comprised the genes expressing the alcohol dehydrogenase (Adh), α-amylase-related protein (Amyrel), aromatic-L-amino-acid decarboxylase (DOPA decarboxylase; Ddc), glycerol-3-phosphate dehydrogenase (Gpdh), and xanthine dehydrogenase (Xdh). The mitochondrial genes included the cytochrome c oxidase subunits I, II, and III (COI, COII and COIII, respectively) and the NADH dehydrogenase subunit 2 (ND2). The ribosomal genes included 28S and the large and small subunits of 12S and 16S (omitting the adjacent tRNAs as they were difficult to align and represented only a small amount of data). For each locus, we aligned the sequences of all species using multiple sequence alignment (MUSCLE) as implemented in MEGA ver. 7.0 (Kumar et al. 2016) and determined its best nucleotide substitution model using jModelTest ver. 2.1.7 (Darriba et al. 2012). The best substitution models were GTR + Γ + I for all nuclear genes and 16S, HKY + Γ + I for the mitochondrial genes and 12S, and HKY for 28S, respectively.

Subsequently, we reconstructed the phylogeny based on Bayesian inference using BEAUTi and BEAST ver. 1.8.3 (Drummond et al. 2012), with unlinked substitution models, a relaxed uncorrelated log-normal clock, and a Yule speciation process. Due to a lack of well-defined fossil dates in our sample of species, and because the absolute timing of speciation events was deemed less important for our analyses than the relative branch lengths, we omitted the time calibration. We ran the Markov Chain Monte Carlo (MCMC) simulation on the CIPRES Science Gateway (< www.phylo.org >; Miller et al. 2010) for 100 million generations, sampling every 10 000th tree. We used Tracer ver. 1.6 (Rambaut and Drummond 2013) to examine the convergence of the Bayesian chain and the stationary states of all parameters, considering effective sample sizes (ESSs) greater than 200 to be adequate. Finally, we generated a maximum clade credibility tree with mean node heights and a 10% burn-in using TreeAnnotator ver. 1.8.3 (Drummond et al. 2012).

In addition to the full *Drosophila* phylogeny, we separately generated a phylogeny for the *Zaprionus* dataset (for which only body lengths and no thorax lengths were available). For these species, adequate sequence coverage was restricted to the Amyrel, COI, COII, and 28S genes, respectively (Supplementary material Appendix 1 Table A2). We used the same procedures as above, except that GTR + Γ + I was the best substitution model for all loci and the MCMC chain was run for only 30 million generations, with a tree sampled every 3000 generations.

Data collection

We measured sex-specific body size as thorax length of field-caught specimens (distance between the tip of the scutellum and the basis of the head, a standard measure) for 56 species of Drosophilidae stored at the Zoological Museum of the Univ. of Zurich. Whenever available, we measured at least 10 individuals per sex per species. We further obtained thorax length data for 111 additional species and data on total body length for 20 *Zaprionus* species from the literature (Supplementary material Appendix 1 Table A3).

For each of our 146 species available in the database TaxoDros (< www.taxodros.uzh.ch/ >), we retrieved the geographic coordinates of every faunistic record. This database

comprises an enormous amount of information on the taxonomy and distribution of more than 6800 species of drosophilids. All coordinates are derived from published data or stem from museum catalogs in which the identification was verified by experts. Although these faunistic data do not stem from standardized collecting schemes but are somewhat haphazard in nature, TaxoDros represents one of the most powerful and comprehensive data sources for any insect taxon. Nonetheless, the sampling coverage might be biased towards certain regions and not be homogeneous across the globe, a common problem when handling such datasets. Thus, we concede that any inference based on these distribution data should be treated with some caution (see Conclusion).

We included only species with at least 20 unique sampling locations in our analyses (removing duplicate and nonsensical localities, resulting in over 25 000 unique coordinates; mean number of coordinates per species: 273.3, SE: 47.9, median: 137). Many drosophilids are distributed globally, but their range is often restricted within latitudinal bands such that they do not occur at the equator. We thus used the mean of the absolute latitudinal distribution to obtain a suitable estimate of the species-specific latitudinal distribution.

Although several major ecogeographic rules describe patterns of latitudinal trait variation, latitude itself remains a compound trait integrating various climatic factors. In this respect, the differential effects of temperature and seasonality are of particular interest. Using climatic data, we tried to decompose latitude into variates related to temperature or seasonality. However, due to the high collinearity among climate variables and latitude, we were unable to use multiple-regression approaches (variance inflation factor always greater than 5 and often greater than 10). When using a principal component analysis (with oblique rotation) to extract the major axes of variation, both temperature and seasonality variables loaded strongly on the first principal component (but in opposite directions, thus mirroring latitude), while all other dimensions did not explain significant proportions of variance and did not show any association to either temperature nor seasonality (Supplementary material Appendix 1 Table A1). For the sake of interpretation (as we were unable to disentangle temperature from seasonality and latitude), we decided to restrict our analyses to latitudinal patterns alone, although future studies disentangling the climate compounds of latitude are clearly desirable.

Thorax length

We analyzed the relationship between log thorax length (mean of male and female values) and median latitude, using phylogenetic generalized linear models (PGLS) as implemented in the R package 'caper' (Orme et al. 2012). We used the sexual dimorphism index (SDI) as an estimate of the strength and direction of SSD as proposed by Lovich and Gibbons (1992). We thus divided the thorax length of the larger sex (usually the female) by the smaller and subtracted 1 from this ratio, which arbitrarily defines the SDI positive if females are the larger sex and negative if males are larger.

Wing size

We retrieved wing-size data for 54 species from Bolstad et al. (2015). These wing sizes represent the square root of wing area derived from outline spline reconstructions. To assess clinal variation in relative wing size, we used PGLS with latitude as the predictor and thorax length as a covariate. In addition, we calculated wing loading, which is typically associated with wing-beat frequency and flight capacity (Pétavy et al. 1997, Frazier et al. 2008). Wing loading is usually defined as some ratio of body mass and wing area, where low values relate to better dispersal capacity as less weight is 'loaded' onto the wing. Because body mass estimates were lacking, we used thorax length^3, which scales well with mass. We analyzed its relationship with latitude using PGLS.

Sexual size dimorphism

To test whether SSD scales iso- or allometrically with body size, we applied phylogenetic reduced major-axis regressions (as implemented in the R package 'phytools': Revell 2012) of log male against log female thorax length across all species (for justification see Fairbairn 1997, Blanckenhorn et al. 2006). Rensch's rule is evident only if the slope of this relationship exceeds one. We repeated the analysis separately for all three major clades of *Drosophila* (*Sophophora* subgenus, *immigrans–tripunctata* radiation, *virilis–repleta* radiation) and the *Zaprionus* spp. data set, for which body size was measured as total body length. To quantify the predictive strength of Rensch's rule in drosophilids, we further calculated r^2 from a PGLS of SDI against log mean size.

Range size

When investigating variation in range size, we considered only species with 20 or more unique records, thus reducing the number of species with sufficient data to 110 (mean number of coordinates per species: 273.3, SE: 47.9; median: 137). To approximate range sizes directly from faunistic records, we derived range-size estimates using α-hulls (as in Gallagher 2016), which are more robust than simple minimum convex polygons, particularly when sampling is haphazard and not standardized (Burgman and Fox 2003). We used the Lambert azimuthal equal-area projection to generate appropriate range-size estimates in km^2 across the globe and restricted these range sizes to actual land masses using the R package 'rangeBuilder' (Davis Rabosky et al. 2016). A PGLS model was used to test for a relationship between log range sizes and latitude (median). In order to test for potential confounding effects of body size and relative wing length (as an estimate of short-distance dispersal), we also performed multiple PGLS regression analyses with thorax length and wing size as covariates. As wing sizes were available for only 54 species, the sample size for this analysis was drastically reduced (note, however, that the number of sampling coordinates per species was greater in this reduced data set: mean: 395.9, SE: 89.7; median: 176).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.b248v> (Rohner et al. 2018).

Results

Thorax length and wing size

Log mean thorax length and wing size increased with median latitude (thorax length: r = 0.23 [95% confidence limits: 0.04, 0.39], λ = 0.97, n = 107, p = 0.019; wing size: r = 0.30 [0.04, 0.51], λ = 0.96, n = 54, p = 0.026; Fig. 1), although the unexplained variation in thorax and wing size was rather large (Fig. 1). Log wing size also showed a positive relationship with latitude when log thorax length was included as a covariate (r = 0.30 [0.03, 0.50], λ = 0.55, n = 54, p = 0.031), suggesting a disproportionate increase in wing size towards high latitudes. Accordingly, wing loading decreased with latitude (r = –0.35 [–0.09, –0.54], λ = 0.00, n = 54, p = 0.009; Fig. 1).

Sexual size dimorphism

Phylogenetic signals in male thorax length (λ = 0.86, p < 0.001), female thorax length (λ = 0.84, p < 0.001), mean body size (λ = 0.85, p < 0.001) and SSD (λ = 0.75, p < 0.001, Fig. 2) suggest phylogenetic inertia of body size and SSD in drosophilids (Fig. 2). When testing Rensch's rule across all species, RMA slopes were significantly steeper than unity ($\beta_{phylRMA}$: 1.10 [1.06, 1.15], p < 0.001, n = 151; Fig. 3), and body size explained 14% of the total variation in SDI. RMA slopes did not significantly differ between radiations (log female size × radiation interaction: $F_{2,114} = 2.31$, p = 0.104), however, when testing Rensch's rule within the three major radiations, we did not find consistent support. Rensch's rule was evident in the *virilis–repleta* radiation ($\beta_{phylRMA}$: 1.08 [1.01, 1.14], p = 0.021, n = 48; Fig. 3), but

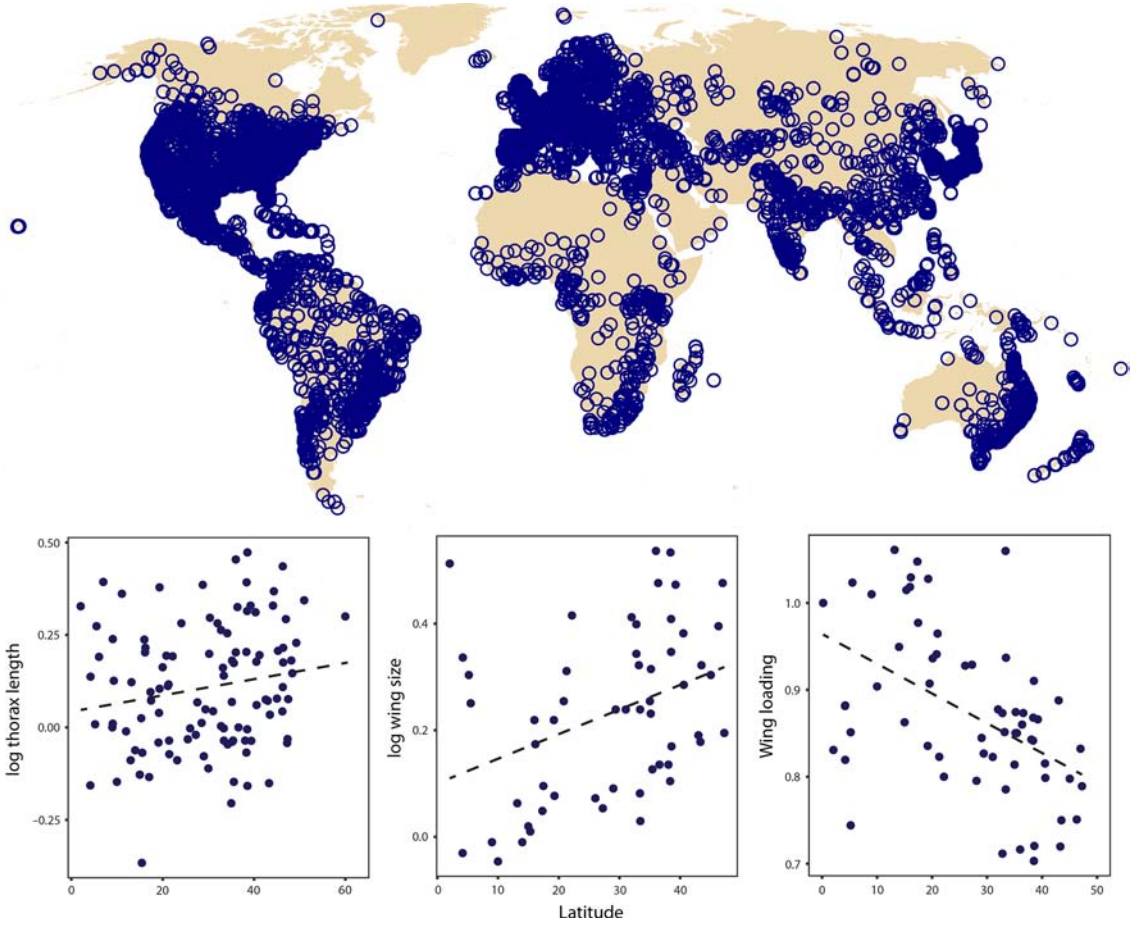

Figure 1. Top: species–specific range size estimates and mean latitudinal distributions were derived from over 25 000 unique sampling locations depicted here. This global dataset was retrieved from TaxoDros, a large and detailed database on taxonomy and diversity of drosophilids (<www.taxodros.uzh.ch/>). Bottom: thorax length and wing size increases with mean absolute latitude, demonstrating a weak interspecific Bergmann cline in drosophilids. Wing size increased more strongly with latitude than thorax length, resulting in lower wing loading (thorax length^3/wing area) towards the poles. These plots showing simple linear regressions are for illustrative purposes only. All analyses were done using PGLS (phylogenetically corrected correlation coefficients are given in the text).

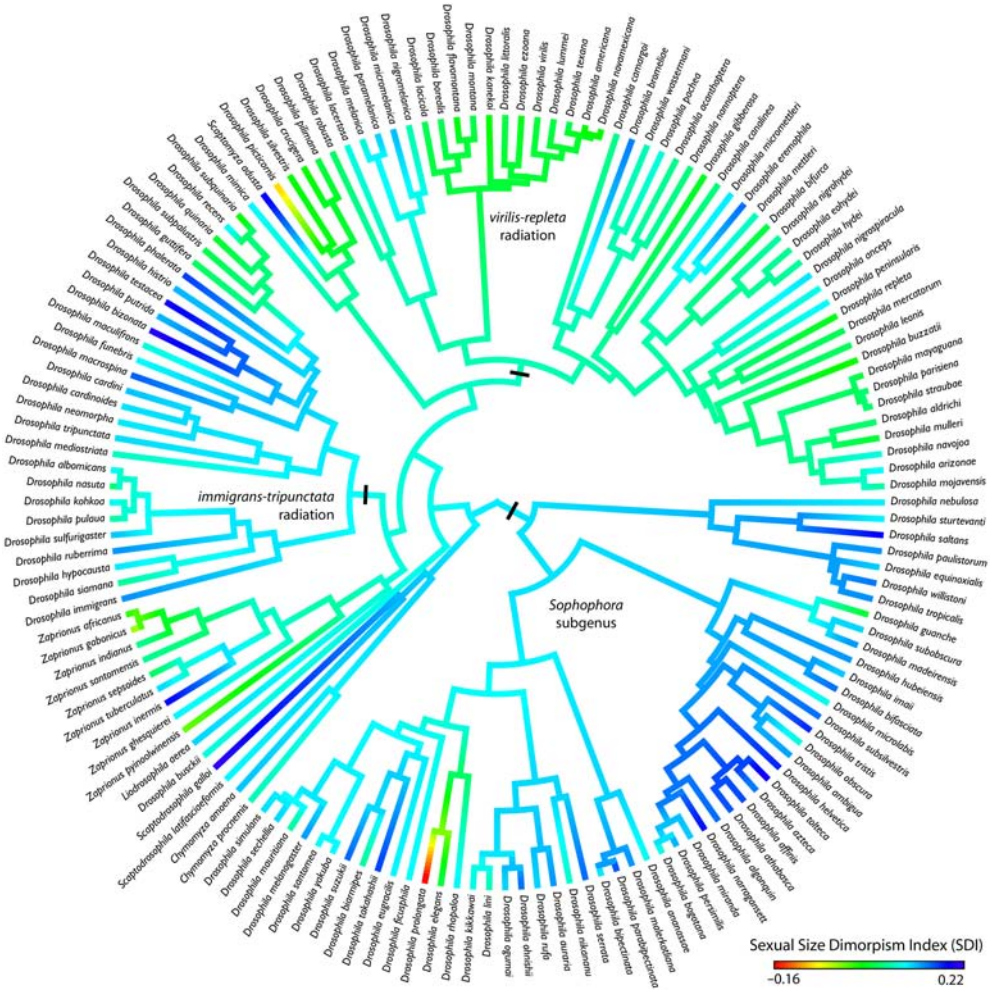

Figure 2. Ancestral state reconstruction of sexual size dimorphism (SDI = (thorax length of larger sex/thorax length of smaller sex) – 1; arbitrarily defined negative if males are the larger sex), for illustration purposes only. SSD shows strong phylogenetic signal and in most species females are the larger sex (positive SDI values). The conspicuous exception is *Drosophila prolongata*, which shows pronounced male-biased SSD.

not in the *immigrans–tripunctata* radiation ($\beta_{phylRMA}$: 1.02 [0.92, 1.13], p = 0.650, n = 22; Fig. 3). In the *Sophophora* subgenus, the RMA slopes were very steep ($\beta_{phylRMA}$: 1.18 [1.06, 1.31], p = 0.002, n = 51; Fig. 3), but this pattern was driven exclusively by *Drosophila prolongata*. This species is by far the largest member of this subgenus and the only one showing male-biased SSD. When excluding *D. prolongata*, Rensch's rule was no longer supported in this clade ($\beta_{phylRMA}$: 0.99 [0.92, 1.08], p = 0.996, n = 50; Fig. 3). The relationship of male and female body length also did not deviate from isometry in *Zaprionus* spp. ($\beta_{phylRMA}$: 1.06 [0.83, 1.28], p = 0.577, n = 16; Fig. 3). There was also no evidence for a correlation between sexual size dimorphism and latitude (r = 0.09, [–0.10, 0.27], λ = 0.67, n = 107, p = 0.369).

Range size

Log range size did not correlate with latitude in the simple linear model using the full data set (r = 0.03 [–0.16, 0.22], λ = 0.03, n = 105, p = 0.777, Fig. 4). However, in a phylogenetic multiple regression including thorax and wing size as additional explanatory variables, range size increased towards the poles (r = 0.37 [0.11, 0.56], p = 0.007, λ = 0.98) whereas thorax and wing length had no effect on range size (thorax: r = 0.10 [–0.17, 0.36], p = 0.469, λ = 0.98; wing size: r = 0.04 [–0.23, 0.30], p = 0.770, λ = 0.98). Note that the data underlying this multiple regression represent only a subset of the data because wing size, thorax length and range extent data were available for only 54 species.

Discussion

Our study of the morphology and global distribution patterns of Drosophilidae lends support to several macroecological phenomena. In accordance with Bergmann's rule, thorax length and wing size increased with latitude, and the same was

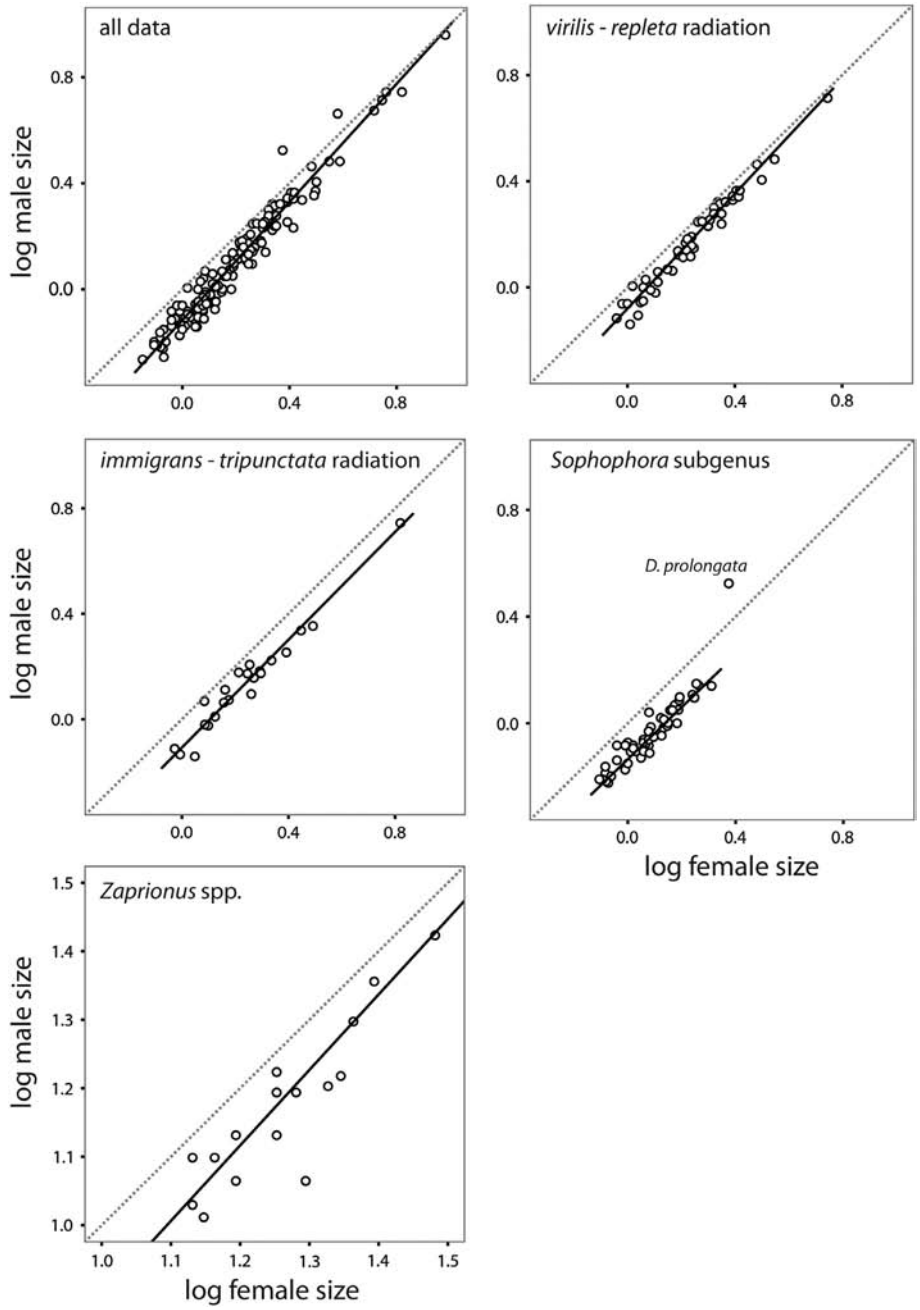

Figure 3. Male size increases more with body size than female size, supporting Rensch's rule for drosophilids. This pattern is however mostly driven by the *virilis–repleta* radiation and absent in all other major clades. For *Zaprionus* spp., only total body length was available while for all other species thorax length was used. Note that regression lines are derived from non-phylogenetic major axis regressions and for illustrative purposes only.

true for relative wing size (contrary to Allen's rule). Our data on SSD also support Rensch's rule overall, but this pattern was mostly driven by the *virilis–repleta* radiation, with weak support in three other major clades. We found no further evidence for a latitudinal cline in SSD. Range size did not vary with latitude across all species of our study, not generally supporting Rapoport's rule. However, when controlling for the potentially confounding effects of body size and shape (and thus reducing our dataset), we found a significant increase in range size with latitude. In the following, we link our results to the ecology and physiology of drosophilids and discuss potential causes and consequences of these macroecological patterns and their apparent idiosyncrasy depending on which species are analyzed.

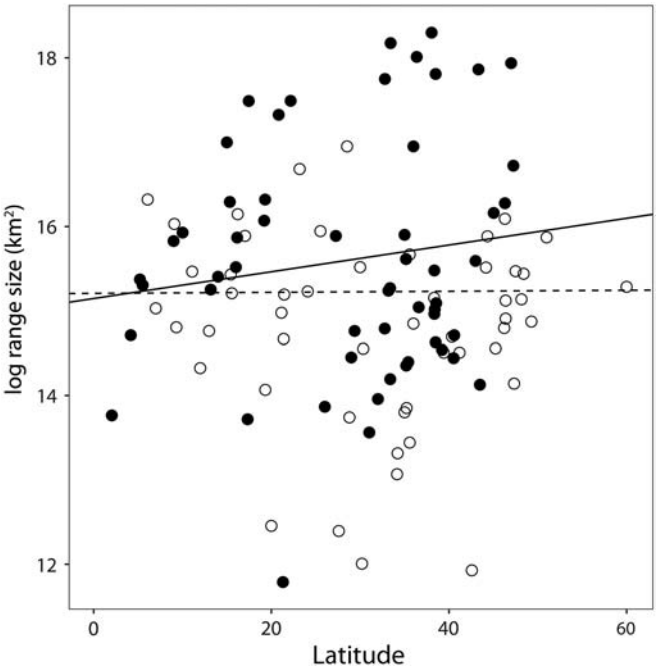

Figure 4. Alpha-hull derived range sizes did not correlate with latitude when all species were analyzed (broken regression line). However, when applying a multiple PGLS regression controlling for thorax length and wing size, range size increased with latitude (solid regression line). Based on merely a limited number of species, this does not seem to be a general pattern across the family, although species for which wing size data were available are dispersed well across the family. The plot shows simple linear regressions for illustrative purposes only. All analyses were done using PGLS (phylogenetically corrected correlation coefficients are given in the text).

Latitudinal effects on body size, shape and range size evolution

Bergmann's rule is thought to be driven by variation in temperature (Atkinson and Sibly 1997, Shelomi 2012), whereas its converse represents an adaptive response to season length (Blanckenhorn and Demont 2004). Drosophilids generally follow a weak positive Bergmann cline, thus at best suggesting only minor effects of temperature and no role of season length in this family. As most drosophilids are small, fast-developing and therefore strongly multivoltine (although some univoltine species and populations exist; Lakovaara et al. 2009), this could be expected (Blanckenhorn and Demont 2004). Compared to the strength of interspecific clinal variation in other insects, the variation explained by latitude in wing ($r^2 = 0.09$) and thorax length ($r^2 = 0.05$) is below average, though not particularly low (cf. r^2 for similar interspecific comparisons from Shelomi (2012): mean = 0.22, median = 0.10, SD = 0.25, n = 18). Given that most individuals measured for this study were collected in the field and not raised under controlled environments, a considerable amount of body size variation must be attributable to phenotypic plasticity. Although this typically also applies to other studies of various taxa, it is possible that we underestimate the strength of the latitudinal pattern.

Even though processes acting within species (sometimes termed neo-Bergmannian rule or James's rule) do not necessarily coincide with among-species patterns (Blackburn et al. 1999), the interspecific clinal variation observed here is consistent with analogous intraspecific variation in drosophilids (Chown and Gaston 2010). Due to this qualitative consistency, it is reasonable to assume a common underlying mechanism. However, following the temperature–size rule (Atkinson 1994), drosophilids tend to grow larger in cool environments in general (Ray 1960), and experimental laboratory rearing would be required to test whether this between-species pattern is driven by evolutionary or purely plastic (i.e. physiological) responses. Note, however, that intra-specific common-garden experiments suggest a strong genetic component (James et al. 1995).

Along with thorax length, wing size increased with absolute latitude, though its steeper increase resulted in disproportionately larger wings at higher latitudes and consequently lower wing loading. Because log wing length showed an isometric relationship with log thorax length across species (evolutionary allometric coefficient derived from a phylogenetic reduced major axis regression: $\beta = 0.94$, $p = 0.528$), allometric scaling relationships cannot explain the relative increase in wing size with latitude. In contrast to warm-blooded animals, in which latitudinal variation in appendage size has been attributed to selection for thermoregulatory efficiency (e.g. reduced bill size in birds: Symonds et al. 2010), such mechanisms seem unlikely to act in insects. Yet, thermoregulation may still be involved in shaping the observed pattern. Being unable to control body temperature endogenously, small insects such as drosophilids regulate body temperature mostly by modifying their behavior (Dillon et al. 2009, Kjærsgaard et al. 2010). Since flight is hampered in the cold and larger wings lower the temperature threshold for take-off (Dillon and Frazier 2006, Frazier et al. 2008), relatively larger wings near the poles could represent an adaptation to large climatic variability or low temperatures (Angelo and Frank 1984, Pivnick and McNeil 1986, Azevedo et al. 1998, Dillon et al. 2009). Such correlations between dispersal capacity and latitude or altitude have been documented in several species (Hassall 2015, Kjærsgaard et al. 2015, Rohner et al. 2015), including latitudinal clines for wing loading in *D. melanogaster* (Azevedo et al. 1998, Klepsatel et al. 2014), and again suggest a common underlying mechanism of intra- and interspecific clines. Yet, greater dispersal capacity may be an essential prerequisite for colonizing habitats at high latitudes in the first place (e.g. following colonization after the last glacial period). Invading less predictable habitats may then in turn promote the evolution of physiological adaptations that are linked to the climate experienced. This alternative explanation seems unlikely, however, given that wing loading did not correlate with range size (see below), and therefore a direct link between colonization success and short-range dispersal ability appears questionable. Increased relative wing size could thus indeed be associated with cold temperature or

increased climatic variability per se and, consequently, a common pattern in small pterygote insects could be expected. Whether this pattern constitutes evidence against Allen's rule as originally formulated or whether such patterns should be discussed in this context at all is certainly debatable, but also not the main point here.

When considering all data, we found no significant correlation between range size and absolute latitude, suggesting no support for Rapoport's rule overall. Our multivariate analysis further suggests that wing size (a proxy for short-range dispersal potential) and thorax length do not confound this relationship, even though both traits have been shown to play major roles in range-size evolution (Malmqvist 2000, Lester et al. 2007, Rundle et al. 2007, Gaston 2009, Swaegers et al. 2014). Surprisingly, the subset of species for which both wing and thorax data were available showed a significant increase of range size with latitude. This discrepancy is unlikely explained by phylogeny, as the species used in the multivariate analysis are well distributed across all major clades (Supplementary material Appendix 1 Fig. A1). At the same time, there was more faunistic information available for these 54 species (based on the number of coordinates), thus likely increasing the precision of our range-size estimation. Alternatively, this deviating pattern could also be caused by the ecology of these particular species. Unlike many other drosophilids (Markow and O'Grady 2006), these species can be cultured easily in the laboratory, so they may be particularly flexible and undemanding in their ecological preferences. If so, their range size might be less affected by ecological barriers such as the distribution of substrate host species. Given this non-random subset and the non-standardized sampling scheme used to derive range size, future research should test these patterns at the global scale.

Sexual size dimorphism and Rensch's rule

Females were the larger sex in nearly all species investigated. There is, however, one particularly conspicuous exception to this trend: *Drosophila prolongata*. This species is not only the largest-bodied species in the *Sophophora* subgenus, but also the only species exhibiting pronounced male-biased sexual size dimorphism (also see Rohner et al. 2017). This species adds further evidence to the notion that there is great potential for rapid evolution of reversed SSD in Diptera, and its apparent association with increased male–male contests (Rohner et al. 2016), which are also common in *D. prolongata* (Kudo et al. 2015). Given its large size and male-biased SSD, *D. prolongata* strongly affected the statistical appraisal of Rensch's rule here (Fig. 3), reemphasizing potential issues with the classic assessment of Rensch's rule when male- and female-biased taxa differ in size (Webb and Freckleton 2007). Nevertheless, the mating system, including the evolutionary drivers of SSD and body size, of *D. prolongata* is likely to be derived, such that this single extraordinary species may obscure rather than testify to Rensch's rule in *Sophophora*.

Although we found support for Rensch's rule across the entire family, this pattern did not hold within some of the major (sub)radiations. Nevertheless, in most cases the reduced major-axis slope between males and females was steeper than one. In fact, empirical research demonstrates frequently that support for Rensch's rule depends strongly on the taxonomic level with considerable variation among closely related clades (Webb and Freckleton 2007). Even if supported in interspecific comparisons, Rensch's rule does not necessarily hold among or within populations of these species (Blanckenhorn et al. 2007a). Within-population variation in SSD is likely driven, at least in part, by ontogenetic processes and thus not necessarily linked to selective forces driving Rensch's rule across species (Teder and Tammaru 2005). In theory, Rensch's rule should nonetheless hold across populations and species. Sexual selection on male size tends to be the strongest and most consistent evolutionary driver of large male size (Székely et al. 2004, Rohner et al. 2016), and Rensch's rule is arguably more prominently supported in taxa with male-biased SSD due to this selective homogeneity (Stuart-Fox 2009). In contrast, fecundity selection mediates female-biased SSD to a much lesser extent than expected, as selection for small male size or other evolutionary scenarios are also common (Pincheira-Donoso and Hunt 2015). Blanckenhorn et al. (2007b) and Huey et al. (2006) found evidence for Rensch's rule in *Drosophila* based on 23 and 42 species, respectively. Our data suggest that this result is robust, but driven mostly by the *virilis–repleta* radiation, while tests in other clades do not support deviations from isometry.

Conclusions

Macroecological rules are sometimes considered to be weak and idiosyncratic, partly because their predictive strength and manifestation varies across taxa, but possibly also because they might be interrelated or confounded. While our comparative analyses largely corroborate previously reported intraspecific patterns for thorax length and wing size, support for Rensch's rule was inconsistent among clades (although slopes did not significantly differ between clades). Moreover, we found no support for Rapoport's rule overall and showed that this pattern is not necessarily associated with thorax length or wing size of high-latitude species. Although entirely correlational, we further suggest that increased relative wing size at higher latitudes may be driven by selection for more efficient flight and thermoregulatory behavior.

We conclude that studying the relationships between several prominent macroecological patterns can shed more light on broad ecogeographic patterns. However, we here found only little evidence for confounding effects. Nevertheless, given that their putative underlying causes are often linked to climatic factors, considering several macroecological patterns simultaneously at minimum permits better interpretation in case of multiple, potentially conflicting trends or hypotheses, as was the case here for wing size and its potential relationship with Allen's rule. Future research should focus on the underlying physiological mechanisms to definitively discern the causes and consequences of various macroecological patterns in Drosophilidae and other taxa.

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Supplementary material (Appendix ECOG-03382 at <www.ecography.org/appendix/ecog-03382>). Appendix 1.

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GLOBAL CHANGE ECOLOGY – ORIGINAL RESEARCH

Largely flat latitudinal life history clines in the dung fly *Sepsis fulgens* across Europe (Diptera: Sepsidae)

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Abstract
Clinal variation in body size and related life history traits is common and has stimulated the postulation of several eco-geographical rules. Whereas some clinal patterns are clearly adaptive, the causes of others remain obscure. We investigated intra-specific body size, development time and female fecundity (egg size and number) clines across 13 European populations of the dung fly *Sepsis fulgens* spanning 20° latitude from southern Italy to Estonia in a genetic common garden approach. Despite very short generation times (ca. 2 weeks at 24 °C), we found a converse Bergmann cline (smaller size at higher latitudes). As development time did not change with latitude (flat cline), integral growth rate thus likely declines towards the pole. At the same time, early fecundity, but not egg size, increased with latitude. Rather than being mediated by seasonal time constraints, the body size reduction in the northernmost flies from Estonia could suggest that these are marginal, edge populations, as when omitting them the body size cline became flat as well. Most of the other sepsid species investigated to date also show flat body size clines, a pattern that strikingly differs from *Drosophila.* We conclude that *S. fulgens* life history traits appear to be shaped by similar environmental pressures and selective mechanisms across Europe, be they adaptive or not. This reiterates the suggestion that body size clines can result as a secondary consequence of selection pressures shaping an entire life history syndrome, rendering them inconsistent and unpredictable in general.

Keywords Body size · Development time · Egg size · Fecundity · Geographic differentiation · Genetic differentiation · Latitudinal cline · Life history

Introduction

The study of large-scale geographic variation in life history traits, first and foremost body size, has a long tradition in evolutionary ecology (Partridge and Coyne 1997; Blackburn et al. 1999; Chown and Gaston 1999; Blanckenhorn and Demont 2004; Shelomi 2012). Body size of closely related species often increases with latitude, known as Bergmann's rule (Bergmann 1847), and corresponding intra-specific trends, sometimes called James' rule (James 1970), also commonly occur. Nevertheless, opposite, so-called converse Bergmann clines also exist (Mousseau 1997; Blanckenhorn and Demont 2004; Shelomi 2012). All these patterns are generally thought to be caused by systematic latitudinal climate variation, although the specific underlying environmental variables and causal mechanisms are frequently unclear. This is because body size is intimately entwined with other life history traits such as juvenile development time and growth rate, which ultimately co-determine final adult body size (Nylin and Gotthard 1998; Blanckenhorn 1999). Moreover, as body size affects most if not all life history and physiological traits of an organism in a concerted syndrome, several reproductive traits (egg size, egg number, etc.) also tend to exhibit geographic clines (e.g. Azevedo

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et al. 1996; Armbruster et al. 2001; Lardies et al. 2010). Though there are many exceptions, higher temperatures usually result in smaller body sizes in ectothermic organisms, an entirely plastic physiological response of unclear adaptive value (named temperature-size rule: Stevenson 1985; Atkinson 1994; van der Have and de Jong 1996; Van Voorhies 1996; Horne et al. 2015, 2018). While temperatures systematically decrease with latitude, season length decreases too, thus constraining an individual's growth period and hence final body size, producing adaptive converse Bergmann size clines especially in large species with long generation times, such as many beetles, grasshoppers and butterflies (smaller towards the poles: Blanckenhorn and Demont 2004; Nygren et al. 2008; Shelomi 2012). Analogous effects are exerted by altitude, albeit at much more condensed spatial scales, such that expected patterns are often not found (Shelomi 2012; Klepsatel et al. 2014; Horne et al. 2018). It follows that several environmental factors (temperature, season length, etc.), more or less independently, act in conjunction on the entire life history syndrome of any particular species, resulting in diverse and potentially intermediate clinal patterns that are ultimately difficult to predict (Blanckenhorn and Demont 2004). More studies of geographic and clinal life history variation are therefore needed.

Although the literature by now boasts many studies on latitudinal clines for a great number of taxa, in insects work on the "model species" *Drosophila* tends to dominate (Shelomi 2012; Schilthuizen and Kellermann 2014; Flatt 2016; Horne et al. 2018). Such strong bias towards *Drosophila* cannot be representative from a biodiversity perspective, especially because Dipterans are very derived phylogenetically (see Misof et al. 2014). This occurs also because small insects, such as *Drosophila*, can be studied as genetic lineages under common garden conditions in the laboratory. Such work therefore tests for evolved, genetic clines, which from an evolutionary point of view is superior to merely phenotypic patterns derived from field-caught specimens, as commonly utilized in macro-ecology (Chown and Gaston 2010). In *Drosophila,* Bergmann clines are the rule within species (e.g. James et al. 1997; Huey et al. 2000; Shelomi 2012; Klepsatel et al. 2014; Fabian et al. 2015; Table 1), and apparently also across species (Rohner et al. 2018a). This can be expected in small species with short generation times, which consequently are multivoltine (multiple generations per year) over most of their distributional range (Blanckenhorn and Demont 2004). By contrast, larger insects such as beetles, grasshoppers or water bugs with typically long development times relative to the available season length often have univoltine life cycles (one generation per year; e.g. Masaki 1967; Blanckenhorn and Fairbairn, 1995; Mousseau 1997; Sota et al. 2000), and thus more likely exhibit converse Bergmann clines because they regularly experience seasonal time limitations forcing them to abbreviate their development at the expense of a reduction in final body size (Blanckenhorn and Demont 2004).

Sepsid "black scavenger" or "dung" flies (Diptera: Sepsidae) are common and widespread worldwide, with many species in the tropics (Ang et al. 2013; see http://sepsidnet-rmbr.nus.edu.sg/ for photographs). On average they are a bit larger than *Drosophila* (3–8 mm body length), and multivoltine throughout most of their range. Sepsids generally depend on decaying organic material for reproduction and larval development, often breeding in livestock faeces (e.g. cow dung; Pont and Meier 2002), a common habitat in agricultural grasslands. In populating ephemeral habitats, sepsids are ecologically similar to *Drosophila* (Blanckenhorn 1999; Rohner et al. 2018a, b). Adult sepsids feed on dung (for protein) and nectar (for energy), and therefore, as *Drosophila*, are anautogenous income breeders, although males (but not females) likely produce their first batch of gametes already during the juvenile phase (Teuschl and Blanckenhorn 2007). Not least because they are easy to rear and have short generation times, sepsid flies are fast becoming model organisms in behaviour, ecology, and evolution (e.g. Teuschl and Blanckenhorn 2007; Puniamoorthy et al. 2009, 2012, 2014; Berger et al. 2013; Rohner et al. 2015; Esperk et al. 2016; Busso and Blanckenhorn 2018). So far, the few studies of latitudinal variation in this group yield inconsistent evidence (summarized in Table 1). Puniamoorthy et al's. (2012) study suggests a Bergmann cline in European but not North American *Sepsis punctum* (see also Dmitriew and Blanckenhorn 2012), and Berger et al. (2013) show a cline in development rate in European *S. punctum* that is consistent with seasonal time constraints operating at higher latitudes (faster in northern Europe). No (i.e. flat) clines are evident in European *S. cynipsea* (Rohner et al. 2016) and *S. thoracica* (Busso and Blanckenhorn 2018).

Sepsis fulgens is one of the most ubiquitous, entirely black sepsid species in Europe about which almost nothing is known so far (Pont and Meier 2002; Fig. 1). We here examine clinal body size, development time and female fecundity (egg number and size) variation of 13 *Sepsis fulgens* populations across roughly 20° latitude from southern Italy to Estonia. Similar to *Drosophila* studies, we took a genetic common garden approach by working with iso-female lines (i.e. the laboratory offspring of single field-caught females) to investigate evolved clines. We additionally assessed the extent of phenotypic plasticity in the above traits by rearing flies at a range of 2–4 laboratory temperatures, thus evaluating the temperature-size rule (Atkinson 1994; Horne et al. 2015, 2018). As this sepsid species is relatively small (body length ca. 4 mm), it features multiple generations per year (e.g. > 8 overlapping generations in lowland Switzerland (pers. obs.); and 2–3 generations per year even at higher latitude in Moscow, Russia: 55.45°N, 37.36°E; Pont and

Table 1 Comparison of clinal studies of Drosophilidae and Sepsidae (Diptera)

Sepsidae	Sex[a]	Geographic range	Temp.	Lat. range	Slope	SE	95% CI low	95% CI hi	No. pops	References
Sepsis cynipsea	M and F	*Europe*	24	43–60	0.00008	0.00050	−0.00091	0.00106	7	Rohner et al. (2016)
Sepsis fulgens	M and F	*Europe*	18	38–60	−0.00849	0.00502	−0.01833	0.00134	11	This study
Sepsis neocynipsea	M and F	*North America*	24	33–44	0.00324	0.00469	−0.00596	0.01243	6	Rohner et al. (2016)
Sepsis punctum	M and F	*Europe*	18	43–60	**0.01471**	0.00493	0.00506	0.02437	7	Berger et al. unpublished
	M and F	*North America*	18	34–46	0.00144	0.00629	−0.01088	0.01376	6	Berger et al. unpublished
Sepsis thoracica	M and F	*Europe*	18	38–60	0.00239	0.01787	−0.03264	0.03741	15	Busso and Blanckenhorn (2018)
Drosophilidae										
Drosophila kikkawai	F	Indian subcontinent	24	7–31	**0.01868**	0.00156	0.01562	0.02174	9	Karan et al. (1998)
Drosophila melanogaster	F	Eurasia	25	38–58	**0.00210**	–	–	–	16	Imasheva et al. (1994)
Drosophila serrata	F	Eastern Australia	25	14–34	**0.11400**	0.00760	0.09910	0.12890	19	Hallas et al. (2002)
	M		25		**0.06900**	0.01550	0.03862	0.09938		
Drosophila simulans	F	Eastern Australia	25	25–42	**0.00337**	0.00100	0.00141	0.00533	10	Arthur et al. (2008)
	M		25		**0.00370**	0.00100	0.00174	0.00566		
Drosophila subobscura	F	Europe	20	37–56	**0.00200**	0.00060	0.00082	0.00318	11	Calboli et al. (2003)
	M		20		**0.00250**	0.00060	0.00132	0.00368		
	F	North America	20	35–50	**0.00190**	0.00070	0.00053	0.00327	11	
	M		20		0.00040	0.00060	−0.00078	0.00158		
	F	South America	20	29–45	**0.00210**	0.00070	0.00073	0.00347	10	
	M		20		**0.00150**	0.00070	0.00013	0.00287		
Zaprionus indianus	F	Indian subcontinent	24	9–31	**0.01190**	0.00230	0.00739	0.01641	10	Karan et al. (2000)
	M		24		**0.01120**	0.00420	0.00297	0.01943		

All slopes refer to wing length (mm/deg. latitude; rescaled from other structural traits for sepsids). Slopes that differ significantly from 0 are in bold

[a]M and F: average slope for both sexes

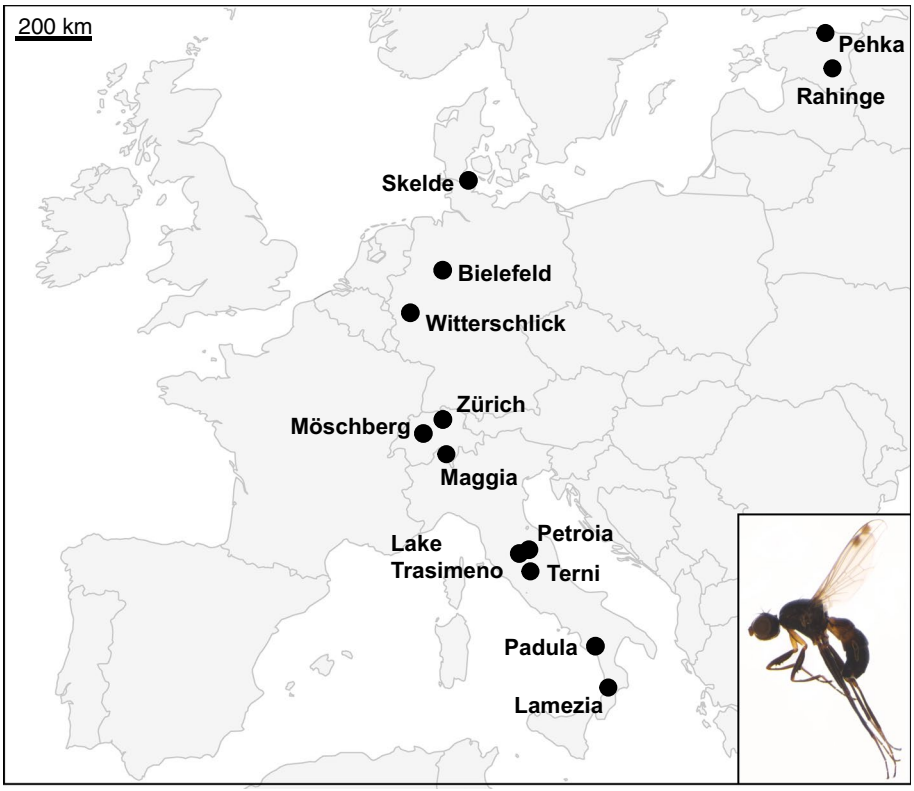

Fig. 1 Map indicating sampling sites of our 13 European *Sepsis fulgens* populations

Meier 2002; cf. Table 2), such that seasonal time limitations would generally not exert strong selection except in the last generation(s) before winter. We thus expected this species to show a Bergmann cline (larger towards the pole), and no countergradient pattern of accelerated development as found for the larger *S. punctum* by Berger et al. (2013; Blanckenhorn and Demont 2004). Consequently, following the expected body size pattern, we also expected either more or larger eggs in populations from higher latitudes.

Methods

Fly cultures and maintenance

Sepsis fulgens Meigen, 1826 (Diptera: Sepsidae) is a eurytopic species occurring throughout Europe, North Africa and the Middle East. The species is commonly found on cow, pig and horse dung, as well as on manure piles (Pont and Meier 2002). Contrary to many sepsids, the species is always entirely black (see http://sepsidnet-rmbr.nus.edu.sg/Sepsis_fulgens.html; Fig. 1).

We studied flies from 13 latitudinal field populations from southern Italy to Estonia (Fig. 1; Table 2 lists some standard climate characteristics of the collections sites). Live wild-caught females from these populations were collected and brought to our laboratory at different times mainly during the seasons 2013 and 2014. Cultures were subsequently kept as iso-female lines (i.e. the descendants of one wild-caught female) for multiple (> 15) generations in the laboratory (ca. 3–4 weeks per generation) before being used in our experiment in 2015. We had several (5–13) replicate families (i.e. iso-female lines) per population (Table 2), each housed in separate 1-l plastic containers that were regularly supplied with fresh cow dung, sugar, and water ad libitum, and kept in a climate chamber at 24 ± 1 °C, 60% relative humidity, and 13:11 h light:dark cycle. Assessing iso-female lines instead of wild-caught females minimises environmental variation influencing the phenotype, thus exposing mainly genetic variation, which remains best preserved in the laboratory by this culturing method.

Laboratory rearing experiments

Two rearing experiments were performed. The first rearing served to analyse body size and development time of both sexes, using 11 populations (cf. Fig. 1): Lamezia, Padula, Terni, Trasimeno, Maggia, Möschberg, Zürich, Bielefeld, Skelde, Rahinge, and Pehka (Table 2). Iso-female lines were provided with fresh dung in their rearing containers, into which multiple females could lay eggs for a duration of up to 4 h. These egg masses were then split randomly among four environments differing in temperature only (5–100 eggs per mass culture dish). The larvae were subsequently allowed to

Table 2 Characteristics and climate conditions at the sampling locations of our 13 populations

Population	Collection (YYYY.M)	No. lines	Latitude	Longitude	Altitude (m)	Winter length[a] (d)	Temperature[b] (°C)			
							Mean annual (bio1)	Mean annual range (bio7)	Mean of warmest quarter (bio10)	Annual precipitation (mm)
Lamezia (I)	2013.4	13	38.92	16.25	244	15	17.6	22.0	24.8	864
Padula (I)	2013.4	13	40.22	15.65	720	128	13.2	22.6	20.6	764
Terni (I)	2012.9	7	42.57	12.62	132	119	14.6	28.1	22.7	853
Lake Trasimeno (I)	2012.9	5	43.13	12.1	258	155	13.3	27.3	21.4	792
Petroia (I)	2014.8	6	43.25	12.55	513	157	12.2	26.0	20.5	936
Maggia (CH)	2013.5	4	46.25	8.7	372	172	10.8	26.6	19.0	1240
Möschberg (CH)	2013.4	11	46.91	7.63	518	200	7.5	25.2	15.4	1067
Zürich (CH)	2013.6	8	47.34	8.55	489	189	9.5	25.8	17.6	1077
Witterschlick (D)	2014.7	6	50.69	7.02	96	186	9.6	23.6	16.9	773
Bielefeld (D)	2013.6	12	52.03	8.53	105	196	8.8	23.1	16.2	833
Skelde (DK)	2013.8	5	54.85	9.73	11	215	8.3	21.5	15.7	750
Rahinge (Est)	2014.6	7	58.37	26.7	72	238	4.9	31.3	15.9	600
Pehka (Est)	2013.5	12	59.48	26.37	57	240	4.7	31.1	15.7	618

[a]Mean number of days below 10 °C (assumed developmental threshold)

[b]Bioclimatic variables 1, 7, 10 from: http://worldclim.org; Hijmans et al. (2005)

develop in overabundant and uniform defrosted cow dung at 60% relative humidity and long photoperiods (≧ 13 h) at constant 12, 18, 24 or 30 °C in 50 ml glass vials. Vials were checked daily for offspring until no more individuals emerged. The thorax length (length of the scutum plus the scutellum) of two randomly chosen offspring males and two offspring females was imaged and measured as an index of body size. The structural outline of the thorax best reflects overall body mass as it contains the important flight muscles.

The second rearing focused on female reproductive traits of eight latitudinal populations: Lamezia, Petroia, Padula, Maggia, Möschberg, Zürich, Witterschlick, and Rahinge (Fig. 1). Male and female flies from any given iso-female line, reared at constant 24 °C, 60% relative humidity and 13 h photoperiod, were paired randomly and subsequently kept in one of two environments differing in temperature only (60% relative humidity and photoperiod ≧ 13 h); for each replicate iso-female line, two pairs were kept in separate 50 ml glass vials with fresh dung and sugar at 18 °C, and two pairs at 30 °C. Every day the dung was checked for eggs. As soon as eggs were laid (=day of first reproduction), they were counted and five of them imaged, and the pair was provided with fresh dung for another day. In case the female laid additional eggs on the second day, we considered them part of the same clutch (as sepsids do not always lay discrete clutches within 24 h). Thereafter, females were frozen and measured. From this experiment, we obtained data on adult age at first reproduction (days), the total number of laid eggs in the first 2 days (i.e. first clutch size, or early fecundity), egg size (length and width, from which egg volume was calculated using the formula for a spheroid: $(1/6)\pi \times \text{length} \times \text{width}^2$). For convenience, fore tibia length was used as an index of female body size; correlations among all structural length traits (including thorax) of *S. fulgens* exceed $r > 0.97$ in both males and females ($n > 40$ individuals).

All images (thorax, eggs, foreleg) were taken with a Leica MZ 12 stereomicroscope and a Leica DC490 digital camera. Measurements were performed using the program tpsDIG2 (© F. J. Rohlf 2010) and the program PAST (© O. Hammer 2003). All analyses were conducted with the software IBM SPSS Statistics 23 (SPSS Inc.).

Results

Development time and body size (thorax length) were analysed with sex and rearing temperature as fixed factors and latitude as a continuous covariate with univariate ANOVAs (general linear models with underlying normal error distribution), using line (i.e. family) means (of typically two individuals per sex) as independent data points (and global error), because this is a genetic study and we were not specifically interested in within-line phenotypic variation. Development time did not change with latitude, i.e. the cline was flat ($F_{1,551} = 0.47$, $p > 0.3$; Fig. 2), with no significant interactions (e.g. temp × latitude: $F_{3,544} = 1.78$, $p = 0.149$; all other interactions $p > 0.2$). In contrast, body size of the flies clearly decreased with latitude ($F_{1,648} = 14.38$, $p < 0.001$; Fig. 2), again revealing no significant interactions (temp × latitude: $F_{3,651} = 1.16$, $p > 0.3$; all other interactions $p > 0.3$). Development time strongly increased as temperature decreased ($F_{3,551} = 477.83$, $p < 0.001$; Fig. 2), while body size was largest at 18 °C, followed by 24 °C, and smallest at both extreme temperatures of 30 and 12 °C ($F_{3,648} = 2.29$, $p = 0.067$, Fig. 2). As usual in insects, females are larger than males in this species ($F_{1,648} = 117.27$, $p < 0.001$). Sexual size dimorphism (calculated as the logarithm of the ratio between female and male thorax length) decreased from cold to warm temperatures ($F_{1,310} = 9.98$, $p = 0.002$); however, this pattern was only due to males being much smaller at 12 °C, as sexual size dimorphism remained constant from 18 to 30 °C. When calculating an analogous index for the development time difference between the sexes, there was no difference between the sexes ($F_{1,201} = 0.08$, $p = 0.146$), and the development time difference did not vary strongly with temperature, although there was a trend of males taking overall longer to develop at cold temperatures ($F_{1,201} = 3.70$, $p = 0.056$; Fig. 2).

The female reproductive trait experiment was similarly analysed with temperature as fixed factor, and latitude, latitude^2 (to test for nonlinearity), and female size (tibia length) as continuous covariates, again using line means (of typically two females) as independent data points. Flies from all populations first reproduced after approximately the same amount of time, but oviposition occurred faster when warmer [18 °C: 6.62 ± 0.073 (SE) days; 30 °C: 4.18 ± 0.076 days], although this difference was not significant ($F_{1,76} = 2.48$, $p = 0.119$). Early fecundity (square-root-transformed number of eggs laid in the first 2 days) was unaffected by rearing temperature ($F_{1,76} = 0.11$, $p = 0.737$) but increased with body size ($F_{1,76} = 7.54$, $p = 0.008$) and latitude ($F_{1,76} = 13.30$, $p < 0.001$), and additionally showed a negative latitude^2 (i.e. hump-shaped, concave) effect ($F_{1,76} = 12.55$, $p = 0.001$), suggesting that the increase in fecundity with latitude could be asymptotic (Fig. 3). In contrast, cube-root-transformed egg volume was not affected by any of these factors (Figs. 3, 4). Consequently, no trade-off between the number and size of laid eggs was found.

Discussion

Despite *S. fulgens'* short generation time (2 weeks at 24 °C, 3 weeks at 18 °C; Fig. 2), we did not find the expected Bergmann size cline (or James' rule) in Europe, but rather

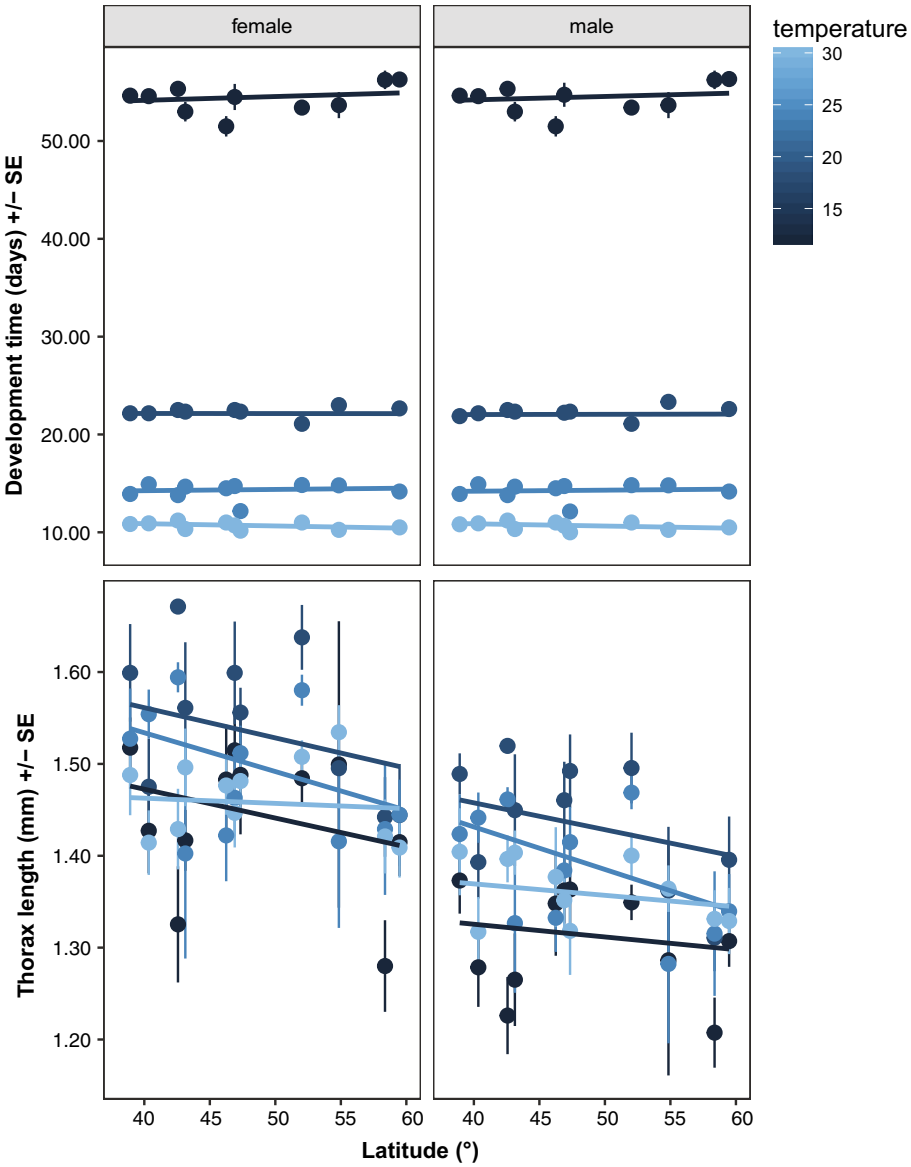

Fig. 2 Population mean ± SE egg-to-adult development time (top) and body size (thorax length; bottom) for females (left) and males (right) of 11 latitudinal *Sepsis fulgens* populations at four constant laboratory temperatures (12, 18, 24, 30 °C)

a converse Bergmann cline (smaller size toward the north pole; cf. Blanckenhorn and Demont 2004). As development time did not change with latitude (Fig. 2), this implies that growth rate does not accelerate towards the pole, although our measurements are restricted to integral measurements of growth, which have been shown to sometimes differ from more precise growth rate measures (Tammaru and Esperk 2007; Rohner et al. 2017). Nevertheless, this result is opposite to what was found for the closely related but larger *S. punctum* (Berger et al. 2013), and also for the much larger yellow dung fly (Blanckenhorn et al. 2018). Rather than being mediated by seasonal time constraints, which would imply an adaptive response (Roff 1980; Kivelä et al. 2011), the size decline in the northernmost flies from Estonia alternatively suggests that these could be marginal, edge populations in terms of environmental factors such as temperature, food, precipitation, or irradiation, etc.; when omitting the two Estonian populations from the analysis (cf. Fig. 1), the European body size cline became flat as well. We therefore conclude that the clinal patterns found here are not necessarily adaptive, as body size, development time, fecundity and egg size are similar across all of Europe, hence presumably shaped by similar environmental pressures everywhere, or alternatively by stabilizing ecological selection relating to these flies' ephemeral food resource (dung). However, we have no direct evidence for the latter, and in the related dung fly *Sepsis cynipsea* overall balancing selection could not be found in an extensive study of one single Swiss population (Blanckenhorn 2007). Unfortunately, we could not obtain *S. fulgens* from more northern sites to verify whether these populations are indeed marginal and/or subject to seasonal time constraints there.

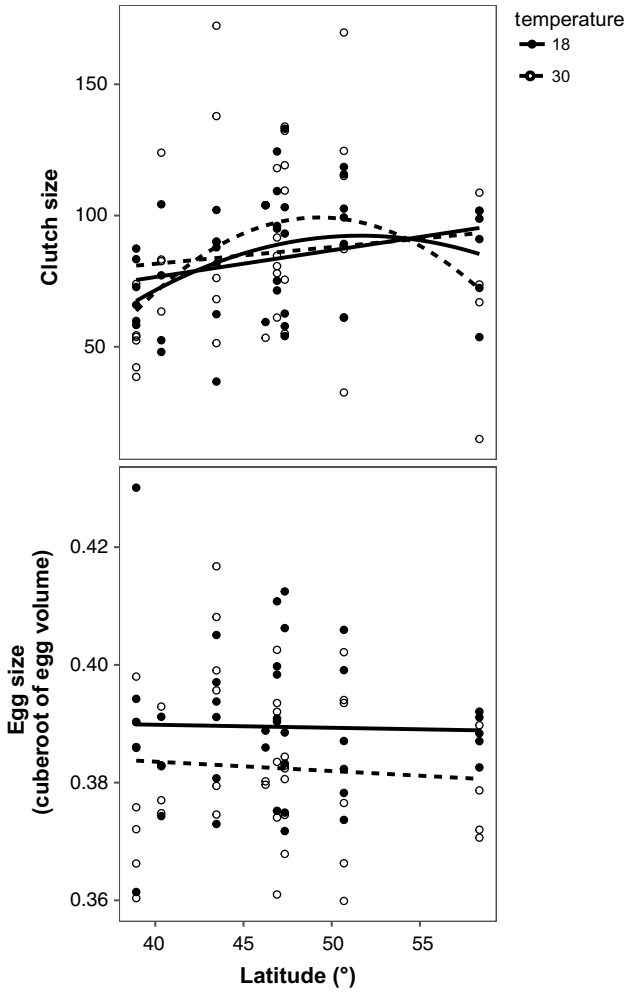

Fig. 3 Line mean number of eggs laid (i.e. first clutch size; top) and size of eggs (bottom) for eight latitudinal populations of lab-reared *Sepsis fulgens* at 18 or 30 °C

Clear body size clines also lack in the related *S. thoracica* and *S. cynipsea* in Europe (Rohner et al. 2016; Busso and Blanckenhorn 2018; Table 1). This situation for sepsids contrasts with that of various *Drosophila* species, the model insects also in evolutionary ecology, which generally show positive Bergmann (or James') clines within species that even have been demonstrated to re-evolve in relatively short time after invasion of a new continent (James et al. 1997; Huey et al. 2000; Zwaan et al. 2000; Klepsatel et al. 2014; Fabian et al. 2015; Flatt 2016; Table 1). Our common garden study implies that all the patterns found for *S. fulgens* (as well as the above-mentioned two other sepsid species) are genetic, i.e. evolved, as is also the case in the listed *Drosophila* studies. As sepsid flies are comparable to *Drosophila* in terms of nutrition ecology, life history, size, dispersal capacity and culturing methods (cf. Blanckenhorn 1999; Rohner et al. 2018a, b), this supports the overall conclusion that the evolution of clinal life history patterns and their underlying mechanisms are complex and not easily predictable in general. In the end, therefore, we are left without being able to identify the mechanisms that mediate the cline differences between the two fly species summarized in Table 1 (cf. Blanckenhorn 1999).

Even though *S. fulgens* flies became smaller, they laid more but not larger eggs at higher latitudes, at least initially between 40° and 50° latitude, beyond which early fecundity declined again in the potentially marginal Estonian populations (Fig. 3). This occurred despite the fact that within populations larger flies produced more eggs (Fig. 4), as is typical in insects (Honek 1993). Berger et al. (2008) showed that the realized fecundity of butterflies can be limited by cool weather, an effect that might select for increased size-specific early fecundity in insects at high latitudes. It could also be that northern flies invest more in female abdomens at the expense of thorax length, but we have no data on abdomen size, which is generally difficult to measure in insects because it is so flexible. Lacking sex by latitude interactions further suggest no sex-specific shifts in investment into the thorax relative to other body parts in females from high latitudes. Egg size also appears to be independent of body size in this species. Derived from Bergmann's rule and the temperature-size-rule (Stevenson 1985; Atkinson 1994; Partridge et al. 1994; van der Have and de Jong 1996; Van Voorhies 1996; Horne et al. 2015), our expectation was that the cooler northern climate would promote the evolution of larger eggs, a plastic response found e.g. in yellow dung flies (Blanckenhorn 2000; Blanckenhorn and Heyland 2004) and *D. melanogaster* (Azevedo et al. 1996), but this was not the case here. Our results also do not support the hypothesis that more extreme climates select for larger eggs providing offspring a competitive advantage during early development (Fox and Czesak 2000). That clutch size increases with latitude while egg size remains unaffected further implies the absence of a size-number trade-off, which is often expected (Smith and Fretwell 1974), perhaps due to seasonal time constraints on this income breeder's life history at higher latitudes (Berger et al. 2008). Again, it seems that clines in reproductive parameters (fecundity and egg size) can turn out equally variable and unpredictable depending on species and environments (cf. Fox and Czesak 2000; e.g. Bauerfeind et al. 2018).

Despite the patterns documented here for *S. fulgens* being heritable, phenotypic plasticity in response to temperature was extensive for development time and body size (Fig. 2), albeit not for reproductive traits (Fig. 3). While development time lengthens strongly and expectedly as temperatures drop, body size did not strictly follow the temperature-size rule in this species (smaller when warmer: Atkinson 1994), as it was maximal at intermediate temperatures of 18 and 24 °C but lower at 12 and 30 °C (Fig. 2). For the related *S. cynipsea,* the lower temperature threshold at which development

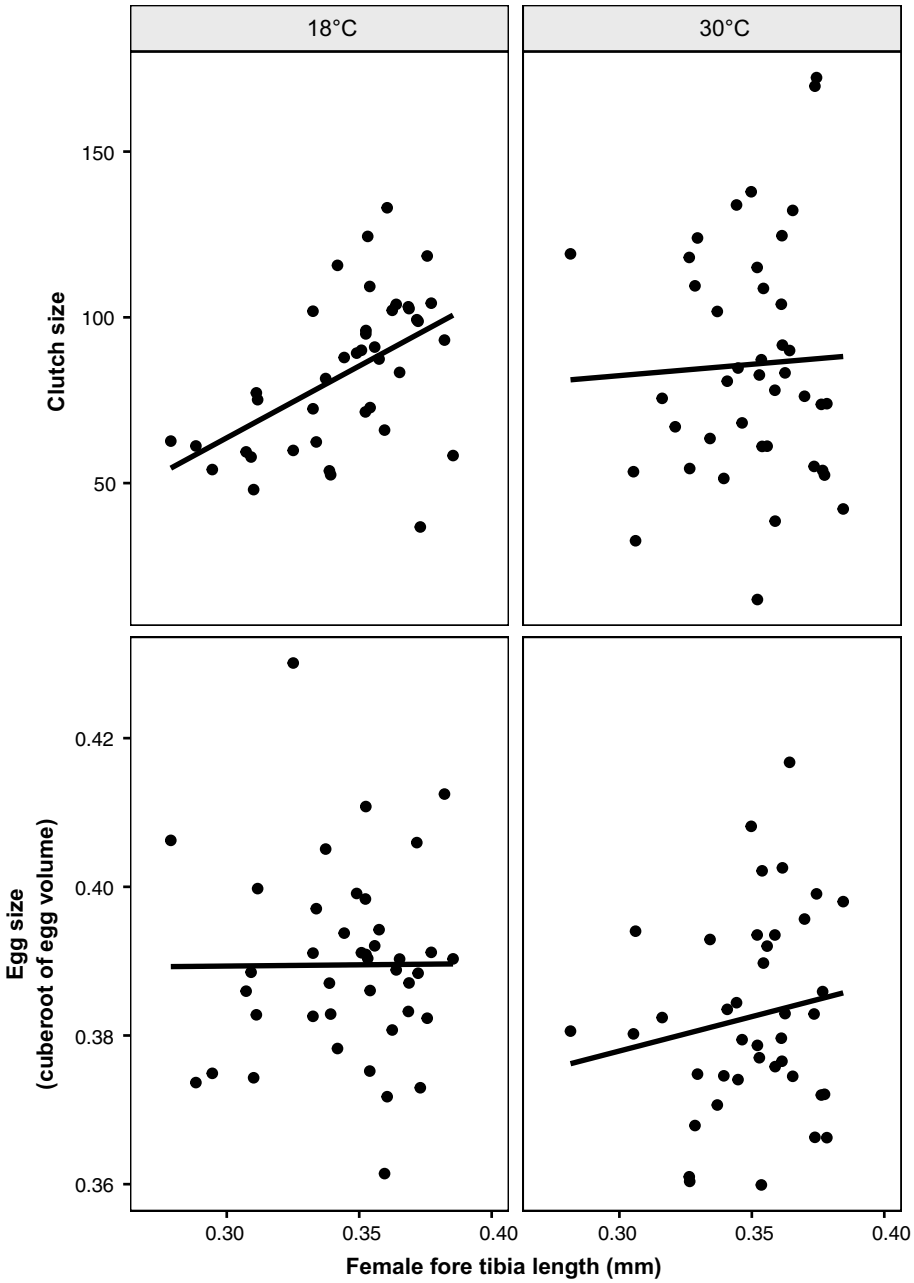

Fig. 4 Line mean number of laid eggs (top) and size of eggs (bottom) of *Sepsis fulgens* females from eight latitudinal populations lab-reared at 18 °C (left) or 30 °C (right) as a function of body size (fore tibia length)

ceases has been estimated to be roughly 9 °C (Blanckenhorn 1999); 12 °C appears to be a marginal temperature for *S. fulgens* as well, at which flies require 55 days to complete development to adulthood (Fig. 2). When reared at temperatures above this threshold, sexual size dimorphism did not vary with temperature, a common pattern in insects (Hirst et al. 2015). The increase in size dimorphism at 12 °C further suggests that the sexes do differ in their temperature-size response at such marginal temperatures: males took longer time to emerge and emerged smaller, so they seem to do worse at cool temperatures. In contrast, rearing temperature of adult females did not affect fecundity here, as is often not the case and thus not expected, whereas egg size was, albeit non-significantly, reduced at 30 °C relative to 18 °C (Fig. 3), as expected on physiological grounds related to processes presumably mediating the temperature-size rule (Van Voorhies 1996; Atkinson and Sibly 1997; Fox and Czesak 2000; Blanckenhorn 2000; Fischer et al. 2003; Blanckenhorn and Heyland 2004; Garrad et al. 2016).

Flat latitudinal clines for body size, development time and/or growth rate in *S. fulgens* as demonstrated here do not necessarily imply that phenotypic clines based on field-caught specimens are also flat. As also shown here, all these traits are considerably plastic, such that lower average temperatures at high latitudes, often combined with low population densities and hence more abundant food per capita,

could suffice to produce Bergmann clines (Blanckenhorn and Demont 2004). Unfortunately, we have no good data on field flies, as we concentrated on collecting live specimens. To convincingly demonstrate phenotypic (i.e. macro-ecological) clines based on field samples in this small and very plastic fly would require numerous specimens sampled systematically at all locations over the season. This is no trivial task. We thus cannot present a phenotypic field cline in addition to the genetic cline. Furthermore, while climate information for our sampling locations is available (Table 2), such data usually do not serve to single out the responsible environmental factors for a given cline, as the crucial parameters, season length and temperature, are typically highly correlated with latitude (and altitude) so that multiple regression approaches necessarily fail. Latitude thus remains the most easily assessable proxy (see Busso and Blanckenhorn 2018; Blanckenhorn et al. 2018).

In conclusion, latitudinal clines in body size, development time and reproductive traits in European *S. fulgens* populations are mostly flat, implying no systematic life history shifts across a wide range of climates. This may relate to these flies being relatively small and ubiquitous, because their substrate (livestock dung) is also ubiquitous in agricultural grasslands that are very common in Europe. Seasonal time constraints therefore probably do not exert strong selection shaping the life history of even the northernmost populations, where these flies still feature multiple generations per season (as, e.g. in Moscow; op. cit.). As sepsid flies are widespread in the tropics (Pont and Meier 2002; Ang et al. 2013), northern European climes likely are secondary, marginal habitats for them, though not in terms of food (i.e. dung) availability. Despite being bad fliers, sepsids are widespread such that gene flow should nevertheless be extensive, hampering local adaptation of populations (Busso and Blanckenhorn 2018). It remains puzzling that sepsids, being ecologically similar to drosophilids also in terms of dispersal capacity and thus gene flow between populations, show very different clinal life history patterns. So, this result reiterates the interpretation that clinal life history patterns are complex and not easily predictable in general, as temperature effects (presumably fostering positive Bergmann clines) and season length effects (fostering converse Bergmann clines) may cancel out, ultimately possibly producing the largely flat clines observed here (Blanckenhorn and Demont 2004).

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Author contribution statement JR, WUB and PTR conceived and designed the study, and contributed all to the statistical analysis and the writing of the manuscript. JR and PTR performed the experiments.

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RESEARCH ARTICLE

Functional Ecology | BRITISH ECOLOGICAL SOCIETY

The evolution of male-biased sexual size dimorphism is associated with increased body size plasticity in males

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Abstract

1. Sexual size dimorphism (SSD) can vary drastically across environments, demonstrating pronounced sex-specific plasticity. In insects, females are usually the larger and more plastic sex. However, the shortage of taxa with male-biased SSD hampers the assessment of whether the greater plasticity in females is driven by selection on size or represents an effect of the female reproductive role. Here, we specifically address the role of sex-specific plasticity of body size in the evolution of SSD reversals to disentangle sex and size effects.
2. We first investigate sex-specific body size plasticity in *Sepsis punctum* and *Sepsis neocynipsea* as two independent cases of intraspecific SSD reversals in sepsid flies. In both species, directional variation in SSD between populations is driven by stronger sexual selection on male size. Using controlled laboratory breeding, we find evidence for sex-specific plasticity and increased condition dependence of male size in populations with male-biased SSD, but not of female size in populations with female-biased SSD.
3. To extend the comparative scope, we next estimate sex-specific body size plasticity in eight additional fly species that differ in the direction of SSD under laboratory conditions. In all species with male-biased SSD we find males to be the more plastic sex, while this was only rarely the case in species with female-biased SSD, thus suggesting a more general trend in Diptera.
4. To examine the generality of this pattern in holometabolous insects, we combine our data with data from the literature in a meta-analysis. Again, male body size tends to be more plastic than female size when males are the larger sex, though female size is now also generally more plastic when females are larger.
5. Our findings indicate that primarily selection on size, rather than the reproductive role per se, drives the evolution of sex-specific body size plasticity. However, sepsid flies, and possibly Diptera in general, show a clear sexual asymmetry with greater male than female plasticity related to SSD, likely driven by strong sexual selection on males. Although further research controlling for phylogenetic and ecological confounding effects is needed, our findings are congruent with theory in suggesting that condition dependence plays a pivotal role in the evolution of sexual size dimorphism.

KEYWORDS
adaptive canalization, condition dependence, Diptera, genic capture, Holometabola, sex-specific phenotypic plasticity, sexual size dimorphism

1 | INTRODUCTION

The strength and type of selection on body size often differ between males and females, owing to their distinct reproductive roles favouring divergent fitness optima (Blanckenhorn, 2000, 2005; Fairbairn, 2013; Fairbairn, Blanckenhorn, & Székely, 2007; Honek, 1993; Shine, 1989). Consequently, sexual size dimorphism (SSD) is widespread across animals and varies greatly among species and sometimes populations (Fairbairn, 2013; Fairbairn et al., 2007).

In insects, females are generally larger than males due to a strong size–fecundity relationship (Honek, 1993). However, despite being rare, male-biased SSD has evolved numerous times independently across the insect phylogeny, often in association with intensified sexual selection on male size and corresponding shifts in the mating system (e.g. Rohner, Blanckenhorn, & Puniamoorthy, 2016). Sexual size dimorphism can differ considerably in its extent, but rarely in its direction (i.e. males or females being the larger sex) among insect species and populations (Rohner et al., 2016; Stillwell, Morse, & Fox, 2007), and often varies strongly across environments due to pronounced sex-specific plasticity in growth and development (Fairbairn, 2005; Fischer & Fiedler, 2001; Stillwell & Fox, 2007). In species with female-biased SSD, females are generally more sensitive to environmental variation (in *c.* 70% of all species studied) and tend to grow disproportionately larger than males along a gradient from poor to good environmental quality, leading to an increase in SSD with body size (Stillwell, Blanckenhorn, Teder, Davidowitz, & Fox, 2010; Teder & Tammaru, 2005). The underlying evolutionary causes of this pattern are poorly understood. Whether the greater plasticity in females is the result of their reproductive role (being female) or of selection on body size (being the larger sex) remains unclear.

For instance, the sexes often differ in their nutritional requirements such that growth can be more strongly affected by nutrient limitation or quality in females than in males (Chapman, Simpson, & Douglas, 2013; Lee, 2010; Moreau, Quiring, Eveleigh, & Bauce, 2003; Stockhoff, 1993), which could cause body size to respond more strongly to environmental variation in females (Teder & Tammaru, 2005). Alternatively, the sex that has its fitness optimum at larger body size may show a stronger response to environmental variation because of greater potential fitness gains with increasing size. In insects, disentangling these alternative mechanisms and assessing whether plasticity is indirectly driven by the reproductive roles or selection on size is inherently challenging because females are the larger sex in the overwhelming majority of species. Studying sex-specific phenotypic plasticity in closely related taxa differing in the direction of SSD can, therefore, prove very useful to differentiate whether sex or size effects drive variation in sex-specific size plasticity. If female size responds more strongly to environmental quality even when females are the smaller sex, the reproductive role is likely to account for sex-specific variation in plasticity independently of size. By contrast, if the level of sex-specific plasticity consistently co-varies with the magnitude and direction of SSD, variation in size plasticity is more likely to result from selection on size.

Sex-specific phenotypic plasticity is ultimately explained by two major alternative hypotheses. First, the adaptive canalization hypothesis (Fairbairn, 2005) predicts decreased plasticity in traits most strongly related to fitness in either sex due to increased developmental canalization by stabilizing selection (or directional selection counteracted by a constraint, Stearns & Kawecki, 1994; Stillwell et al., 2010). Alternatively, the condition dependence hypothesis posits that plasticity increases by strong directional selection for resource-use efficiency and so captures interactive genetic and environmental effects (Amend et al., 2013; Bonduriansky, 2007a; Oudin, Bonduriansky, & Rundle, 2015; Rowe & Houle, 1996). Although these two hypotheses predict opposing patterns of plasticity, differentiating between them is not straightforward. For example, female body size may be more plastic than male size due to directional selection on female size, but strong stabilizing selection on male size (or any other trait associated with body size such as growth rate or development time: Wiklund & Fagerstrom, 1977) could lead to an identical pattern. A rigorous test of these hypotheses thus requires knowledge of the selective forces driving the system, data on multiple traits, and/or comparative data that may reveal which sex evolved a heightened degree of body size plasticity.

Here, we address the role of sex-specific body size plasticity in the evolution of male-biased SSD in insects by integrating approaches at three different taxonomic levels: (1) within two species of black scavenger flies (Diptera: Sepsidae) that convergently evolved intraspecific reversals of SSD; (2) among fly species dispersed across the higher Diptera clade; and (3) in a meta-analysis across Holometabola. Sepsid flies are particularly well suited to study such patterns due to considerable SSD variation in both magnitude and direction even among closely related species and populations. Male-biased SSD evolved independently several times across the family, and the direction of SSD further varies within species. *Sepsis neocynipsea* and *Sepsis punctum* show directional variation in SSD between North American and European populations. In *S. neocynipsea*, males are larger than females in North America, while females are the larger sex in Europe (Rohner et al., 2016). In *S. punctum*, this pattern is reversed across the same continents (Dmitriew & Blanckenhorn, 2012, 2014; Puniamoorthy, Schafer, & Blanckenhorn, 2012). In both species, male-biased SSD is derived and driven by enhanced sexual selection on male size, whereas the intensity of fecundity selection on female size does not differ between male- and female-biased populations (Puniamoorthy et al., 2012; Rohner et al., 2016).

Taking advantage of these two independent microevolutionary systems with known underlying selective drivers, we conducted controlled laboratory experiments to identify which sex shows greater body size plasticity, and to test competing hypotheses based on our understanding of the underlying selective forces. If the reproductive role of females is the main driver of increased plasticity, we expected females to show greater plasticity in general, even in species with male-biased SSD. In contrast, if the larger sex is also the more plastic sex irrespective of whether males or females are larger, selection on size is likely to be a more important force. Decreased plasticity of the larger sex, in contrast, would suggest a role of adaptive canalization driven by stabilizing selection and/or directional selection, with body size otherwise being constrained at its upper limit (Fairbairn, 2005). Finally, lack of any sex-specific plasticity (i.e. constant SSD across environments) would suggest that either its evolution is constrained, or that selection pressures counterbalance and thus canalize variation in SSD across environments.

Previous research has demonstrated that different environmental variables can have disparate effects on sex-specific plasticity. Whereas sex-specific plasticity is common when food quality or quantity is manipulated (Stillwell et al., 2010; Teder & Tammaru, 2005), SSD does not seem to vary consistently with temperature across arthropods (Hirst, Horne, & Atkinson, 2015). However, in Diptera, females tend to decrease more strongly in size than males with increasing temperature (leading to a reduction in female-biased SSD with increasing temperature: Hirst et al., 2015). We, therefore, here not only manipulated food quantity, but also rearing temperature to test whether results can be generalized across multiple environmental variables.

Our second goal was to understand the evolution of sex-specific body size plasticity more broadly. To this end, we conducted a comparative study by gathering detailed data for three additional dipterans with male-biased SSD (*Sepsis lateralis*, *Drosophila prolongata*, *Scathophaga stercoraria*) and five closely related fly species with female-biased SSD (*Sepsis cynipsea*, *Sepsis fulgens*, *Drosophila melanogaster*, *Drosophila rhopaloa*, *Musca domestica*). We thus tested whether the association between sex-specific body size plasticity and SSD in *S. neocynipsea* and *S. punctum* extends to these additional flies in a more general pattern across the Diptera. Finally, we analysed published data on species with contrasting SSD in a meta-analysis to test for an even broader pattern among holometabolous insects. Integrating our results from the intraspecific case studies with the comparative Dipteran and holometabolous insect data, we discuss the general role of condition dependence, sex and body size in the evolution of sexual size dimorphism and reversals thereof.

2 | MATERIALS AND METHODS

2.1 | Intraspecific variation in sex-specific plasticity of *S. neocynipsea* and *S. punctum*

Outbred laboratory populations of *S. neocynipsea* and *S. punctum* were established using offspring of at least 10 wild-caught, gravid females of European (both species: Zurich, Switzerland) and North American (*S. neocynipsea*: Montana, USA; *S. punctum*: Georgia, USA) origin following standard laboratory protocols (Puniamoorthy et al., 2012). These populations were cultured for several generations at densities of *c.* 200–300 individuals.

For egg collection, each laboratory population was provided with a petri dish filled with cow dung for oviposition. After 3–4 hr, depending on the number of eggs laid, this dish was removed and incubated at 18°C for 24 hr. Thereafter, the freshly hatched first-instar larvae were retrieved from the dung by rinsing it with tap water and removing larvae using a fine brush. These larvae were then randomly assigned to different environmental treatments. To maximize environmental variation, we used a factorial design (three food treatments × two temperatures) for each population. In the unlimited food treatment, we provided 10 larvae with 6 g of standardized dung in a rectangular plastic dish. We mimicked natural food limitation by filling the lids of 1.5-ml Eppendorf tubes with dung and placing either a single larva (intermediate food limitation: 0.3 g per individual) or 10 larvae (strong food limitation: 0.03 g per individual) into it. To prevent desiccation, we placed all dishes into glass vials fitted with wet cotton. For the intermediate food treatment, we combined several Eppendorf tube lids in one glass vial, whereas in the two remaining treatments only one dish/Eppendorf lid was placed per vial. These glass vials were treated as independent experimental replicates (random effect). For each population and each food x temperature treatment, we generated at least three such replicates. When no adults emerged, we repeated the experiment to increase our sample size. The experimental procedure in these temporal blocks was identical, but we statistically accounted for this random block effect nevertheless (see below). Vials were maintained in climate chambers at either 15 or 28°C. Upon emergence, adults were sexed and frozen. To estimate body size, we removed the hind legs of each fly and mounted them on glass slides in Euparal, which were subsequently photographed and measured to determine the mean length of both hind tibiae. Note that hind tibia length correlates strongly with other measures of body size, and the sexes do not differ in the allometric relationship of tibia in relation to thorax length (Table S1). Hind tibia length thus well represents overall size. Furthermore, studies of primarily sexual selection in the close relative *S. cynipsea* (Blanckenhorn, Kraushaar, Teuschl, & Reim, 2004) show no specific morphological trait targeted by selection, but rather "overall body size." Hind tibia length is thus unlikely to be a direct target of selection, except indirectly via body size effects.

To assess sex-specific plasticity within populations, we used linear mixed models with (mean) hind tibia length as a function of sex, temperature and food quantity, including all interactions. All non-significant interactions were discarded, except for the sex × food quantity and the sex × temperature interactions, which were our focus. We used replicates (the identity of the glass vial used for incubation) and experimental block (date on which replicates were set up) as random effects. In addition, we also formally tested whether the sexes differ in their body size response to food quantity between continents. To this end, we tested for a food quantity × population interaction for males and females of each species separately. A significant interaction term would suggest population differentiation in the sex-specific slope

of the reaction norm (body size as response to food), whereas a significant population main effect would suggest a shift in the intercept. Replicates, temporal blocks, as well as temperatures were added as random effects in these models. All analyses were conducted in R (R Core Team, 2016) using the package LME4 (Bates, Machler, Bolker, & Walker, 2015).

2.2 | Interspecific variation in sex-specific plasticity in Diptera

To examine sex-specific plasticity beyond our two focal species *S. punctum* and *S. neocynipsea*, we also lab reared several closely related dipteran species that differ in the direction of SSD. These additional species included three other *Sepsis* spp., two with female-biased SSD (*S. cynipsea* and *S. fulgens*) and one with male-biased SSD (*S. lateralis*). We further studied two other clades of Diptera showing both directions of SSD. In the *Drosophila* clade, *D. prolongata* exhibits male-biased SSD, and *D. rhopaloa* and *D. melanogaster* female-biased SSD (data for the last species derived from the literature: Miller (1964)). The second clade included two calyptrate Diptera, with *M. domestica* exhibiting female-biased and *S. stercoraria* male-biased SSD (data on the latter from (Blanckenhorn, Pemberton, Bussiere, Roembke, & Floate, 2010). Given that these species dwell on various substrates and are adapted to different ecological niches, we cannot directly compare environmental treatments across species. We, therefore, did not use identical treatments across species but crossed different larval densities (1–60 individuals per container) with various amounts of food (0.3–100 g) and temperatures (15–30°C; see Table S2) separately for each species. Each species thus experienced different food and temperature treatments, mimicking a strong environmental gradient within species. Although the conditions differed between species, this did not hamper our main goal, the comparison of body size variation between the sexes within species, which were of course always reared under identical environmental conditions. *Musca domestica* and *Sepsis* spp. were reared on cow dung, *D. prolongata* and *D. rhopaloa* on standard *Drosophila* medium. As traditionally different proxies of size are used for different dipteran species, we used thorax length or log adult weight for all drosophilids and *M. domestica* but hind tibia length for all sepsids and *S. stercoraria*. We are aware that using different body size surrogates may to some extent confound the interspecific comparison. However, our research mainly focussed on between-sex comparisons within species such that the trait used to estimate body size was secondary and unlikely to greatly confound variation in SSD (because species with both male- and female-biased SSD were scored for tibia as well as thorax length).

2.3 | Data analysis

To assess sex-specific plasticity, we calculated the sex-specific mean body size for each environmental replicate (temperature × larval density) per species and regressed log(male size) against log(female size) across these replicates in reduced major-axis regressions (RMA), as is standard (Fairbairn, 2007). RMA slopes equal the ratio of the standard deviations of the *y*- and *x*-axes. Hence, slopes deviating from unity in these regressions indicate sex-specific plasticity, with slopes >1 suggesting greater variation in male size (*y*-axis) across environmental conditions and slopes <1 greater female variation (*x*-axis). Because such ratios produce asymmetric effect-size distributions, we used the natural logarithm of the RMA slopes as index for the strength and direction of sex-specific plasticity (producing a symmetrical effect-size distribution).

We further quantified the strength and direction of SSD, either using independent datasets of our own or data retrieved from the literature (flies were raised at overabundant food in all cases), by calculating the sexual dimorphism index (SDI) as proposed by Lovich and Gibbons (1992). To this end, we divided the size of the larger sex by that of the smaller and subtracted 1 from this ratio, and arbitrarily assigned positive signs when females are the larger sex and negative ones when males are larger. To control for phylogenetic non-independence we used phylogenetic generalized linear models (PGLS) as implemented in the R-package *caper* (Orme et al., 2013), using log(RMA) as the response and SDI as the explanatory variable. Since detailed phylogenetic information was lacking, we constructed a cladogram derived from published literature (Setoguchi et al., 2014; Wiegmann et al., 2011; Zhao, Annie, Amrita, Yi, & Rudolf, 2013) and set all branch lengths to one. Note that we included our above data for North American and European *S. neocynipsea* and *S. punctum* populations in these analyses as well.

2.4 | Meta-analysis across Holometabola

To test for a general pattern in holometabolous insects, we gathered data from the literature, focussing, where possible, on closely related species pairs or triplets that differ in their direction of SSD (even if they do not represent sister species). In general, we followed the procedure of Teder and Tammaru (2005) and accepted studies in which diet, food amount, larval crowding or ant attendance (for some lycaenid butterflies) were manipulated. Further, we only considered studies presenting data for at least four environmental treatment levels for females and males separately. Adult weights at eclosion as well as pupal weights were accepted as body size estimates, although the former was preferred if both were available. The nature of environmental manipulations was very diverse, including different host species for parasitoids and herbivores, or various manipulations of food quantity or quality for other species (Table S3). Such treatments thus cannot be compared directly across species. To assess sex-specific plasticity quantitatively, we therefore again regressed species-specific log(RMA) slopes across environmental treatments (as above) against SDI. As independent body size data were lacking for most species, the mean SDI across environments was calculated for each species and used to estimate species-specific SSD. To account for the precision of RMA estimates per species, which increases with the number of independent treatment levels, our linear regression was weighted by the number of treatments within species. This approach further corrects, at least to some extent, for the different magnitudes of the environmental gradient used in different studies.

TABLE 1 Using hind tibia length as a proxy for overall body size, we found that food quantity had a pronounced effect on size in all species/populations. However, the sexes only differed in their response to food quantity in North American populations of *Sepsis neocynipsea* and European populations of *Sepsis punctum*, both of which show male-biased sexual size dimorphism (SSD). This suggests an association between SSD reversals (i.e. the evolution of male-biased SSD) and increased condition dependence in male size. Statistics are derived from general mixed models including replicate and experimental block as random effects

	Sepsis neocynipsea						*Sepsis punctum*					
	North America male-biased SSD			Europe female-biased SSD			North America female-biased SSD			Europe male-biased SSD		
Effect	***df***	***F***	***p***	***df***	***F***	***p***	***df***	***F***	***p***	***df***	***F***	***p***
Sex	1,103.07	4.68	.03	1,115.84	8.97	<.001	1,268.53	4.26	.04	1,154.17	17.8	<.001
Food quantity	2,41.05	86.6	<.001	2,65.14	171	<.001	2,86.68	102	<.001	2,43.64	79.9	<.001
Temperature	1,37.8	0	.99	1,74.03	11.2	<.001	1,112.55	0.32	.57	1,50.06	3.09	.08
Sex × food quantity	2,102.74	5.14	.01	2,119.22	0.11	.9	2,245.93	0.18	.83	2,150.98	6.31	<.001
Sex × temperature	1,114.64	1.48	.23	1,127.1	1.58	.21	1,248.09	5.32	.02	1,156.88	0.28	.6
Temperature × food quantity	2,38.72	5.8	.01	2,74.26	21.8	<.001						

3 | RESULTS

3.1 | Intraspecific variation in sex-specific plasticity of *S. neocynipsea* and *S. punctum*

Food quantity had a strong positive effect on hind tibia length in all populations studied (Table 1). Crucially, the effect of food quantity differed between the sexes in North American (NA) *S. neocynipsea* as well as in European (EU) *S. punctum* (sex × food quantity interaction in Table 1). In these populations, the sexes were essentially monomorphic at low food quantity but males increased more strongly in size with increasing food quantity, leading to considerable male-biased SSD under ample food conditions (Figure 1). Both independent intraspecific SSD reversals thus feature increased plasticity in males, while this pattern was absent in the sister populations with female-biased SSD (sex × food quantity interaction not significant in Table 1; Figure 1). In addition, we found no differences in the response of female body size to food quantity between continents in either *S. neocynipsea* (continent × food quantity interaction: $F_{1,39.07} = 0.68$, $p = .413$; Table S4) or *S. punctum* ($F_{1,89.80} = 2.14$, $p = .148$; Table S4). In contrast, males differed in their plastic response to food quantity between continents (continent × food quantity interaction: *S. neocynipsea*: $F_{1,17.44} = 9.49$, $p = .006$; *S. punctum*: $F_{1,46.30} = 37.13$, $p < .001$; Table S4), suggesting that the differences in sex-specific plasticity observed among populations are driven by variation in male body size plasticity alone.

The effect of food quantity on tibia length further differed between temperatures in both populations of *S. neocynipsea* (food quantity × temperature interaction; Table 1), although this did not affect SSD (because the sex × food quality × temperature three-way interactions were non-significant throughout and hence removed; *S. neocynipsea* NA: $F_{2,83.75} = 0.45$, $p = .640$; EU: $F_{2,110.67} = 0.88$, $p = .420$; *S. punctum* NA: $F_{2,156.51} = 0.79$, $p = .460$; EU: $F_{2,14.03} = .09$, $p = .910$). The sexes differed in their reaction to temperature only in North American *S. punctum* (sex × temperature interaction in Table 1). In this population, female tibia length increased more with decreasing temperature than in males, suggesting that female body size is more plastic in response to temperature.

3.2 | Interspecific variation in sex-specific plasticity in Diptera

Log(RMA) slopes were always steeper in taxa with male-biased SSD than in those with female-biased SSD (i.e. males are more plastic than females when they are the larger sex; Table 2). Log(RMA) slopes decreased significantly with the degree of female bias in SSD (PGLS: $F_{1,10} = 8.03$, $p = .018$, $r = -0.67$, $\lambda = 0.00$ [95% CI: 0.00–0.89], slope = −0.91; Figure 2a), demonstrating that taxa with relatively larger males have steeper RMA slopes. Since the reversed pattern was also observed when females were larger than males (lower right quadrant in Figure 2a), the larger sex generally seems to show heightened plasticity.

3.3 | Meta-analysis across holometabolous insects

Combining our own data with data from the literature, we obtained information on sex-specific plasticity for a total of 43 species (Coleoptera: eight species; Diptera: 16 species; Hymenoptera: four species; Lepidoptera: 15 species; see Tables S3 and S5). All these data are restricted to Holometabola, as studies of other insect groups did not fit our requirements. The number of environmental treatments per species varied from 4 to 23 (median: 7, $M \pm SD$: 7.3 ± 3.7). When averaging SDI across environments, 21 species showed female-biased SSD whereas males were the larger sex in 22 species (SDI ranging from −0.41 in *D. prolongata* to 0.32 in the cowpea seed beetle *Callosobruchus maculatus*).

Across all 43 species, log(RMA) showed a negative relationship with SDI (weighted least-squares regression: $t_{1,41} = -2.48$, $p = .017$, slope = −0.52; Figure 2b). Since the intercept is close to zero (estimate = −0.003, $t_{1,41} = -0.12$, $p = .907$), males tend to be more plastic

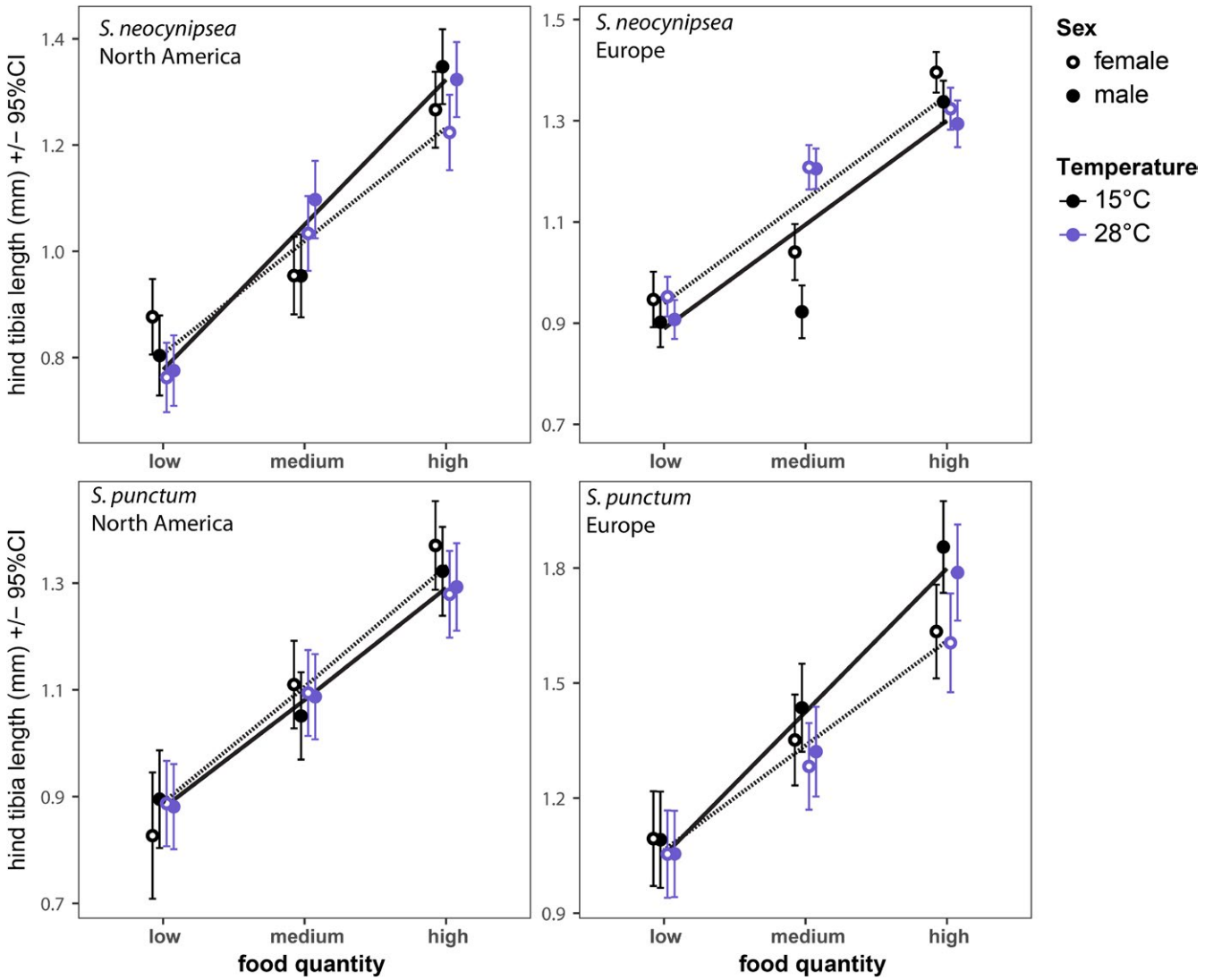

FIGURE 1 Mean hind tibia length of sepsid fly populations reared at three food (dung) quantities and two temperatures. Males increase more strongly in size with environmental quality in North American *Sepsis neocynipsea* and European *Sepsis punctum* (solid lines), the populations in which males are larger than females. In contrast, the sexes do not differ in their plastic response in populations with female-biased sexual size dimorphism (dotted lines). This pattern qualitatively holds in *S. punctum* and *S. neocynipsea*, although the latter shows a weaker sex-by-environment interaction. *M* ± *SE* estimates represent model parameters and their associated errors; random variation among blocks and replicates is thus accounted for. For simplicity, we only show average sizes across temperatures, but raw data are shown in Figure S1

than females in species with male-biased SSD, and females tend to be more plastic than males in species with female-biased SSD. When restricting the analysis to previously available data from the literature, this relationship was qualitatively similar but no longer statistically significant (weighed least-squares regression: $t_{1,29} = -1.05$, $p = .302$, slope = −0.29).

4 | DISCUSSION

Our study demonstrates an association between sex-specific body size plasticity and the strength and direction of SSD across holometabolous insects (Figure 2). In general, the larger sex tends to be more plastic in response to environmental factors, thus being more condition dependent. This result is consistent with the hypothesis that sex-specific plasticity is driven mainly by selection on size rather than selection associated with the reproductive role (i.e. being male or female). However, at least in the sepsid flies, size plasticity is not entirely symmetrical with regard to sex. Stronger condition dependence in males is likely mediated by strong sexual selection (Bonduriansky, 2007a, 2007b; Figure 1, 2a, Table 2). We discuss potential evolutionary and ecological drivers of these patterns and their implications for the study of body size and SSD evolution.

4.1 | Intraspecific variation in sex-specific plasticity in *Sepsis*

In North American *S. neocynipsea* and European *S. punctum*, the derived male-biased SSD is associated with increased plasticity in males

TABLE 2 Reduced Major Axis (RMA) slopes of log male size against log female size with various numbers of replicates (*n*) reflecting a large environmental gradient. All slopes are significantly greater than unity in taxa with male-biased sexual size dimorphism (SSD), whereas for female-biased taxa slopes vary around 1. We used the sexual dimorphism index (SDI), a standardized ratio, as an estimate of the direction and strength of SSD (Lovich & Gibbons, 1992), derived from independent datasets in which flies were raised with ad libitum food at benign temperature. Means (95% CI) are given for the two SSD groups in bold italic

Taxon	Authority	*n*	R^2	RMA slope	*p*	SDI estimate
Male-biased SSD						
Drosophila prolongata	Singh & Gupta, 1977	17	.88	**1.37**	**.003**	−0.3
Scathophaga stercoraria[a]	Linnaeus, 1758	5	.99	**1.2**	**.045**	−0.25
Sepsis punctum EU	Fabricius, 1794	21	.85	**1.24**	**.021**	−0.07
Sepsis neocynipsea NA	Melander & Spuler, 1917	17	.96	**1.14**	**.015**	−0.04
Sepsis lateralis	Wiedemann, 1830	15	.91	**1.23**	**.026**	−0.03
				1.236 (0.074)		***−0.138 (0.111)***
Female-biased SSD						
Sepsis punctum NA	Fabricius, 1794	31	.94	0.96	.406	0.03
Sepsis fulgens	Meigen, 1826	30	.71	1.01	.925	0.04
Sepsis neocynipsea EU	Melander & Spuler, 1917	16	.83	1.09	.459	0.05
Musca domestica	Linnaeus, 1758	21	.81	1.06	.557	0.07
Sepsis cynipsea	Linnaeus, 1758	26	.97	**0.87**	**.001**	0.07
Drosophila rhopaloa	Bock & Wheeler, 1972	15	.79	1.14	.324	0.11
Drosophila melanogaster[b]	Meigen, 1830	11	.94	**0.69**	**.001**	0.13
				0.974 (0.114)		***0.071 (0.027)***

[a]Blanckenhorn et al. (2010).
[b]Miller (1964).

(significant sex-by-food quantity interaction, RMA slope >1), while conspecific female-biased populations show no sex-specific plasticity (sex-by-food quantity interaction not significant in Table 1; RMA slope not different from 1 in Table 2), a clear sexual asymmetry in condition dependence. In populations with larger males, SSD was absent in stressful environments but increased gradually with environmental quality (Figure 1). This pattern can be caused either by increased condition dependence in males or developmental canalization in females. As male body size plasticity in response to food availability differs between continents, while female plasticity does not (see Table S4), population differentiation must be caused by variation in male plasticity only, suggesting evolution of increased condition dependence in males in populations with male-biased SSD. This fits well with previous studies demonstrating that the intensity of sexual selection on male size is stronger in the male-biased populations while there is no indication for differences in fecundity selection on female size among populations (Puniamoorthy et al., 2012; Rohner et al., 2016). Hence, there is no evidence for stronger (stabilizing) selection potentially canalizing female size in male-biased populations only. We thus attribute the greater male plasticity in male-biased populations to increased directional selection on male size, consequently arguing in favour of the condition dependence hypothesis and rejecting the canalization hypothesis (Bonduriansky, 2007a; Fairbairn, 2005).

In contrast to food availability, temperature did not strongly affect sex-specific plasticity. This finding is common in insects (Hirst et al., 2015). We found a significant temperature effect on SSD only in North American *S. punctum*, in which female size declined more strongly than male size from low to high temperature, whereas European populations responded more plastically to food (Table 1). Hirst et al. (2015) also found that in Diptera SSD unusually declines with temperature. So it is possible that the extent of sex-specific plasticity and condition dependence varies with the taxon and the environmental variable in question. According to the temperature-size rule (Atkinson, 1994; Atkinson & Sibly, 1997), low temperatures generally produce larger individuals through physiological responses. However, this size increase does not necessarily co-vary with environmental quality (Atkinson & Sibly, 1997), and it is thus unclear whether this response means increased condition (dependence), which currently hampers a functional interpretation.

Originally proposed to explain variation in ornament size via genic capture, condition dependence is predicted to link genome-wide genetic quality of an individual to the expression of its secondary sexual traits in a given environment (Rowe & Houle, 1996). This opportunistic mechanism should allow individuals to invest optimally in costly traits under resource limitation, flexibly trading fitness gains in sexual selection against viability (or any other) costs. Theory thus predicts a tight association between sexual dimorphism and condition dependence (Bonduriansky, 2007a, 2007b). In *Sepsis* and insects more generally, large size entails viability costs due to prolonged development time and/or increased growth rate (Blanckenhorn, 2000, 2009; Teder, 2014). In both sepsids and drosophilids, for example, males take longer to develop than females, possibly related to male gonad

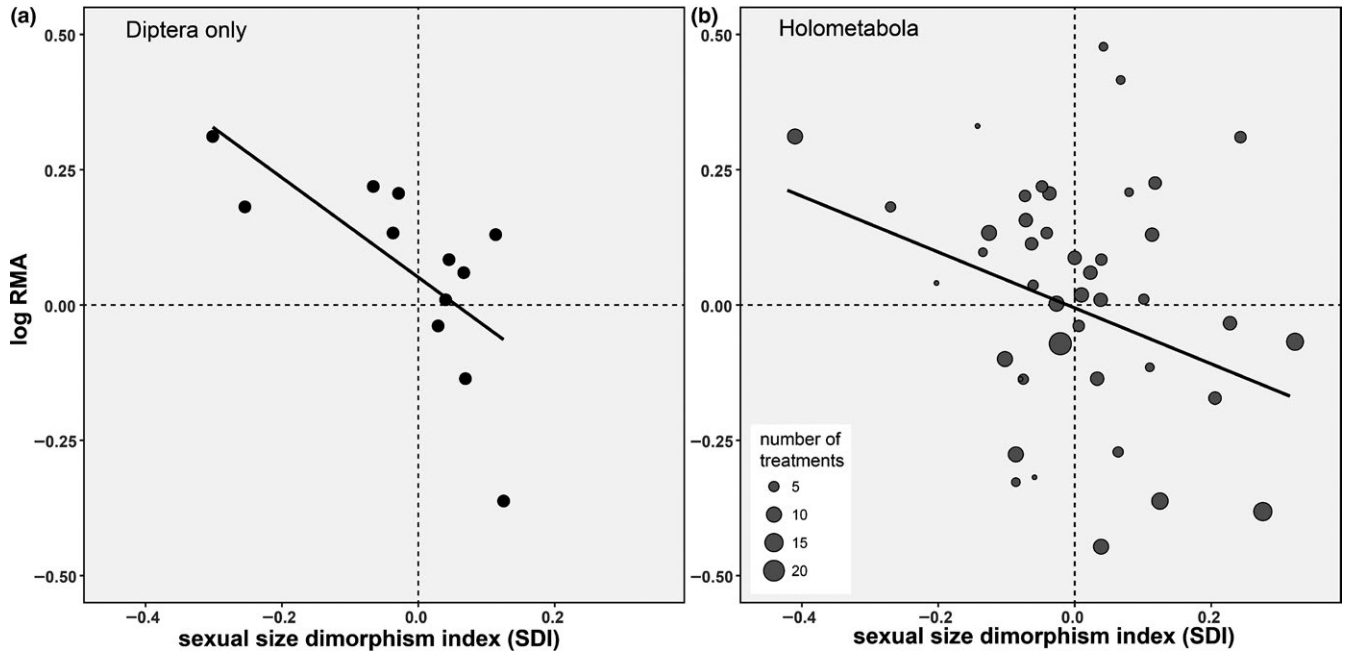

FIGURE 2 The relative plasticity of males (estimated by log(RMA) slopes) increases with the relative size of males (decreasing sexual dimorphism index [SDI]) in Diptera (a) and Holometabola in general (b). This suggests that the evolution of male-biased sexual size dimorphism (SSD) is associated with an increase in male plasticity. Log(RMA) slopes larger than zero indicate that males are more responsive to environmental variation (RMA slope = *SD*(males)/*SD*(females)), while females are more plastic if this slope is less than zero. To quantify SSD, we divided the size of the larger sex by that of the smaller and subtracted 1 from this ratio, and arbitrarily assigned positive signs when females are the larger sex and negative ones when males are larger (=SDI). While SDI of independent datasets were used in the analysis for Diptera only, we used the mean SDI across environments for the Holometabola in b). The trend line in b) gives the weighted linear regression using the number of treatments as weights (as indicated by the size of points)

or gamete development (Blanckenhorn et al., 2007; Rohner et al., 2016), so the costs of growing large at limited food are expected to be greater for males. This sex difference should be amplified if habitats are ephemeral and/or when sexual selection favouring large male size is particularly strong. When facing serious food limitation, males may therefore not be able to grow larger by prolonging growth due to severe mortality risks. Instead, they may shorten their larval development and emerge as adults earlier but at a smaller size. These small males are not favoured by sexual selection, but by reaching the adult stage they at least maintain some potential for direct fitness. Such a "bail-out" strategy in response to food limitation has been found in several dung-dwelling beetles (Shafiei, Moczek, & Nijhout, 2001) and flies (Blanckenhorn, 1999), and likely explains the evolution of greater male body size plasticity in *S. neocynipsea* and *S. punctum* populations with male-biased SSD. Selection on adult male size could thus indirectly lead to the evolution of condition dependence in larval growth rate and developmental time.

In contrast, the absence of sex-specific plasticity in populations or species with female-biased SSD is not congruent with the condition dependence hypothesis (sex × food quantity interaction not significant). When females are the larger sex, as is most common in ectotherms, it is equally reasonable to assume that females would benefit to a greater extent than males from investing in body size at limited resources, but they do not show increased plasticity in our dataset. In females, condition dependence should be driven primarily by fecundity selection. Fecundity selection tends to be generally weaker than sexual selection on males in sepsids and other species, specifically also in the two species studied here (Puniamoorthy et al., 2012; Rohner et al., 2016), and further tends to asymptote at the largest body sizes in *S. cynipsea* (Blanckenhorn 2007). Perhaps as a consequence, female-biased SSD is relatively weak in *S. neocynipsea* and *S. punctum*, such that the absence of sex-specific plasticity here may be explained by rather weak divergent selection on body size, in which case other selective pressures may obscure any patterns (see also below). Alternatively, this lack of sex-specific plasticity might be confined to tibia length and not necessarily apply to other estimates of body size, which we, however, consider unlikely because tibia length well reflects body size in many fly species (Table S1).

4.2 | Interspecific variation in sex-specific plasticity in Diptera

In extension of the above argument, the magnitude and direction of SSD co-varied with sex-specific size plasticity among several species of flies, again suggesting that females are not inherently more responsive to environmental quality (e.g. due to their particular nutritional needs), but that the larger sex is generally more plastic. As predicted by theory (Bonduriansky, 2007a, 2007b), this suggests a pivotal role of condition dependence in the evolution of male-biased SSD, and of SSD in general.

It is important to note, however, that while RMA slopes of species with male-biased SSD are always significantly steeper than unity and often strongly so, species with female-biased SSD frequently do not show significant sex-specific size plasticity (RMA slopes not significantly shallower than unity in Table 2). As argued above, this may well be caused by the relatively weak SSD of female-biased species and the concomitant low levels of divergent selection that could be counteracted by other forms of selection. This finding corroborates the results of Teder and Tammaru (2005) showing that female size plasticity is more likely to exceed that of males as the magnitude of female-biased SSD increases. Nevertheless, irrespective of whether intra-specific RMA slopes significantly deviate from unity, the overall interspecific pattern for the Diptera covered here suggests a rather strong and sexually not entirely symmetric (inverse) relationship between condition dependence and SSD (Figure 2a).

4.3 | A general pattern in Holometabola?

Our quantitative meta-analysis adds further evidence to the notion that the larger sex tends to be more plastic (Teder & Tammaru, 2005), thus suggesting a general trend at least across the Holometabola (Figure 2). This result should be treated with some caution, however, as it was not quite statistically significant when excluding the dipterans, although the pattern itself persisted, again suggesting that Diptera are somehow different. Since our experimental rearing specifically aimed at covering extreme environments including severely limited and overabundant resource availabilities that should well cover the range experienced in nature (Blanckenhorn, 2009), our RMA slopes should adequately estimate the pattern with low biological error. The differences between Diptera and the rest of Holometabola might therefore merely be quantitative (as opposed to qualitative), with the larger sex generally being more plastic. Alternatively, however, as demonstrated by Hirst et al. (2015), patterns of sex-specific plasticity can differ among insect orders (see also Teder & Tammaru, 2005), likely caused by shared phylogenetic relatedness, life histories or habitats.

So why might sepsids, and possibly other Diptera, differ from other insects in the impact of sex-specific condition dependence on SSD expression? We can only speculate at this point. All flies studied here depend on ephemeral resources for reproduction and development. Since condition dependence is expected to be more common when resources are strongly limited, which regularly applies to the short-lived and unpredictable resources of dung flies and perhaps also *Drosophila* (Blanckenhorn, 1999, 2009), this might explain the discrepancies between Diptera and other Holometabola found here. Further data on species dwelling in other substrates are therefore required to test how common the phenomenon is, although it may not explain the sexual asymmetry in sex-specific plasticity. Alternatively, Blanckenhorn et al. (2007) also uncovered an asymmetric sex-specific pattern in that females of several insect groups, including sepsids and drosophilids but also water bugs, grow faster than males. This suggests lower viability costs for females counteracting the generally weaker fecundity selection on female size (relative to the typically stronger sexual selection on male size: Blanckenhorn et al., 2007; Rohner et al., 2016). At least in sepsids and drosophilids, male costs are presumably exerted by the time and energy consuming production of male gonads and gametes (Blanckenhorn et al., 2007; Lupold et al., 2016), provoking stronger viability counter-selection in males. Increased investment into body and organ size thus appears generally costlier in males, but also more rewarding as sexual selection on male size tends to be stronger than fecundity selection on female size. The evolution of stronger condition dependence in males compared to females, allowing to flexibly counterbalance costs depending on environmental circumstances, thus seems to have some adaptive value (Bonduriansky, 2007a, 2007b; Rowe & Houle, 1996).

In conclusion, our study of species varying in the direction of SSD revealed that male-biased SSD is associated with increased phenotypic plasticity of males in (higher) Diptera if not all Holometabola. We corroborate theoretical predictions by demonstrating that condition dependence, particularly in males through sexual selection, plays a pivotal role in generating both quantitative and qualitative variation in sexual size dimorphism, within as well as across species. It remains yet unclear, however, whether this pattern extends to other phylogenetic clades and ecological guilds, or even beyond insects (c.f. Blanckenhorn et al., 2007). Further data for other taxonomic groups covering a wide range of different life histories and ecological adaptations will be needed to evaluate the generality of this phenomenon. Given the complex developmental patterns generating sex-specific plasticity (Stillwell & Davidowitz, 2010), particular attention should be paid to the underlying physiological and genetic mechanisms for a more comprehensive understanding of the evolution of SSD, plasticity and condition dependence (Davidowitz, 2016; Rohner, Blanckenhorn, and Schäfer, 2017).

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AUTHORS' CONTRIBUTIONS

All authors conceived the ideas and designed methodology; P.T.R., S.L. and T.T. collected the data; P.T.R. analysed the data; P.T.R. and W.U.B. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.f6r60 (Rohner, Teder, et al. 2017).

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A comparative study of the role of sex-specific condition dependence in the evolution of sexually dimorphic traits

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Key words: allometry, Diptera, life history evolution, mating system, scaling relationships, Sepsidae, sexual selection, sexual size dimorphisms, phenotypic

Abstract

Sexual selection can displace traits acting as ornaments or armaments from their viability optimum in one sex, ultimately giving rise to sexual dimorphism. The degree of dimorphism should hence not only mirror the strength of sexual selection, but also the net viability costs and benefits of trait maintenance at equilibrium. The ability of organisms to bear exaggerated traits will depend on their condition. More sexually dimorphic traits should therefore also exhibit greater sex differences in condition dependence. While this has been shown to apply among traits within species, condition dependence and sexual dimorphism are also expected to correlate across the phylogeny. We investigated and quantified this prediction within and across 11 (sub)species of black scavenger flies that vary in their mating system. When estimating sex-specific condition dependence for seven sexual and non-sexual traits that vary in their sexual dimorphism, we not only found a positive relationship between the sex difference in allometric slopes (as our measure of condition dependence) and relative trait exaggeration among traits within species, but also across species for those traits expected to be under sexual selection in males. We additionally show species with more pronounced male aggression to have relatively larger and more condition-dependent male fore and mid legs. Our comparative study suggests a common genetic/developmental basis of sexual dimorphism and sex-specific plasticity that apparently evolves across the phylogeny, and that the evolution of trait size consistently alters scaling relationships and thus contributes to the allometric variation of sexual armaments or ornaments in animals.

Introduction

The magnitude, type or form of selection typically varies between sexes. Thus, directional sexual selection often drives the evolution of exaggerated male secondary sexual traits that serve as ornaments or armaments by shifting phenotypes away from their viability fitness optimum, which may differ markedly from the female optimum of the same trait (Andersson 1994; Fairbairn et al. 2007). Reflecting the evolutionary net outcome of various sex-specific natural and sexual selection pressures, such sexually antagonistic selection ultimately gives rise to sexual dimorphism (Blanckenhorn 2005, 2007; Preziosi & Fairbairn 2000; Price 1984). If sexual selection is driving dimorphism, and assuming the between-sex genetic correlation does not impose major constraints, the displacement from the viability selection optimum reflects the net costs and benefits of (exaggerated) trait expression (which may not be the case if dimorphism is due to ecological character displacement, e.g. Temeles et al. 2000, Shine 1989, 1991). The capacity of organisms to bear particular traits typically varies to the extent that only individuals in good condition, i.e. those with access to more metabolic resources (Rowe & Houle 1996), will be able to afford expressing a certain degree of trait exaggeration that then can act as indicator of their intrinsic quality. Traits under strong directional selection, for instance via female choice, are therefore expected to show a heightened degree of condition dependence (Iwasa & Pomiankowski 1991, 1999). This is because condition dependence, a form of phenotypic plasticity linking an individual's genome-wide genetic quality to trait expression under a given amount of resources (Rowe & Houle 1996), allows to flexibly trade off survival costs that arise through trait exaggeration with the corresponding reproductive benefits. (Think of the antlers of a male deer to be regrown every year to an impressive size depending on the available extrinsic resources and the intrinsic quality, i.e. mass

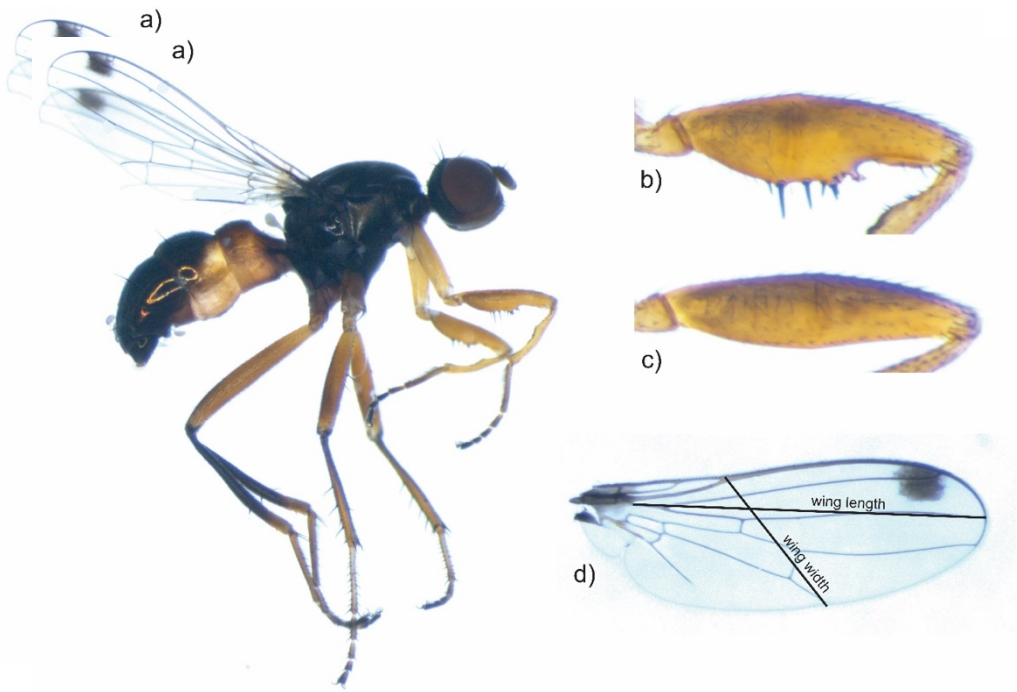

Figure 1: General morphology of Sepsis punctum (a). The male fore femur (b) exhibits pronounced protrusions and spines that are completely absent in females (c). Measurements for wing length and width are indicated in (d).

or size of the individual, relative to the antlers of the female). If individuals in good condition enjoy larger marginal benefits, they should show greater trait investment (Bonduriansky & Day 2003, Johnstone et al 2009). Sexually antagonistic directional selection should hence not only drive the evolution of sexual dimorphisms, but also evoke sex-specific variation in the extent of condition dependence, if only because both are driven by selection. Consequently, although formal theory to this effect is currently lacking (but see Iwasa & Pomiankowski 1991), the sex showing phenotypic exaggeration for a given trait should also show heightened condition dependence, and sexual dimorphism itself should become dependent on condition (Bonduriansky 2007a,b; Bonduriansky & Rowe 2005; Bonduriansky & Day 2003).

The predicted association between dimorphism and condition dependence for morphological traits has received great attention in vertebrate and invertebrate species with very conspicuous (sex-specific) armaments or ornaments (Cotton et al. 2004b; Tomkins et al. 2010). Such work was historically grounded in early comparative studies of solitary and social primates, ungulates and birds attempting to understand the evolution of sexual dimorphism by their underlying developmental processes (Blanckenhorn et al. 2007; Bro-Jørgensen 2007; Jarman 1983; Leigh 1992; Teather & Weatherhead 1994). This was also of interest to developmental biologists and geneticists because sex-specific condition dependence represents a form of sex-lmited epistasis, which could also resolve between-sex genetic correlations that would otherwise hamper the establishment of sexual dimorphisms (Bonduriansky 2007a,b). However, many previous studies are limited to few traits, lack non-sexual "control traits" in the same or opposite sex, or provide data for only one sex. Studying merely few traits not only poses difficulties in terms of statistical power, but might also be unrepresentative, hence hampering evaluation of the generality of putative relationships. To fully understand and quantitatively assess the predicted co-variation between dimorphism and condition dependence, multiple sexual and non-sexual traits need to be studied analogously in both sexes.

The rather few experimental studies explicitly investigating the relationship between dimorphism and sex-specific condition dependence suggest a positive correlation (Bonduriansky 2007a, Bonduriansky & Rowe 2005, Oudin et al. 2015, Punzalan et al. 2008, Johns et al. 2014, but see Cayetano & Bonduriansky 2015). Transcriptomic studies in *Drosophila melanogaster* (Wyman et al. 2010) and the dung beetle *Onthophagus taurus* (Ledon-Rettig & Moczek 2016) also suggest a link between sexual dimorphism and condition

dependence. Patterns among traits within species thus appear to be consistent. However, based on the same argument, sex-specific condition dependence and sexual dimorphism are also predicted to correlate across species, provided that other selective ecological pressures are similar and intersexual genetic correlations or morphological integration do not pose major constraints (Bonduriansky 2007b). Thus, in species subjected to an increased degree of sex-specific directional selection on a given trait (e.g. following mating system evolution), sexual dimorphism should become amplified, and so should the benefit of condition dependence (Rowe & Houle 1996). However, this prediction remains underexplored at the interspecific level, and the existing studies are often limited to few traits of primarily stalk-eyed flies (Baker & Wilkinson 2001; Cotton et al. 2004a,b,c; Wilkinson & Taper 1999, Kawano 2004, Simmons & Tomkins 1996) hampering our understanding of whether and how the relationship between dimorphism and condition dependence can evolve.

Testing this prediction is complicated because estimating condition dependence is difficult in many organisms. This is because traits often develop for long time and/or irregularly grow merely during specific and non-overlapping life stages or seasons. While this is a pervasive issue in many species with indeterminate growth, such as many vertebrates or crustaceans, condition dependent trait expression can be much more readily studied in morphological traits of holometabolous insects. Holometabola develop essentially all their adult tissue during metamorphosis, with the entire energy budget determined at the onset of metamorphosis. If reared in a standardized environment where only the amount of food is manipulated, the overall size of the pupa, and consequently the adult, thus estimates the total available metabolic resources, i.e. condition. Because different adult traits develop simultaneously and compete for resources during metamorphosis, the relationship of relative trait size to overall body size in the adult –the static allometric coefficient (Cheverud 1982; Klingenberg & Zimmermann 1992)– therefore well estimates the degree of dependency of trait expression on condition (Shingleton & Frankino 2018). This is further supported by studies of the developmental underpinnings of allometry. Proximately, variation in static allometric slopes among traits has been linked to variation in the sensitivity of various organ primordia responding to varying levels of insulin-like peptides (ILP's). Because the insulin-signaling pathway links nutrition to growth, ILP-sensitivity mirrors the dependency of the growth of a specific structure to the nutritional status (Emlen et al. 2012; Shingleton & Frankino 2018; Tang et al. 2011). Therefore, static trait allometries of holometabolous insects, and likely many other organisms as well, should reflect variation in the responsiveness of trait growth to condition, i.e. condition dependence, such that steeper allometries imply greater allocation of resources to a given trait with size. Note however that although we here use allometric slopes as a measure of condition dependence, the two concepts are not neces-

Table 1: Taxonomic authority, population origin and the direction and extent of sexual size dimorphism in the eleven taxa studied here (data from Rohner et al. 2016). The sexual size dimorphisms index (SDI), a standardized ratio (Fairbairn et al. 2007), was calculated by dividing size of the larger sex by the smaller and subtracting 1 from this ratio. The index arbitrarily defines SDI as positive if females are larger, and negative if males are larger.

Species	Origin	Sexual size dimorphism (SSD)	SDI
Saltella sphondylii (Schrank, 1803)	Zürich, Switzerland	female-biased	0.03
Sepsis cynipsea (Linnaeus, 1758)	Zürich, Switzerland	female-biased	0.07
Sepsis duplicata Haliday, 1838	Zürich, Switzerland	female-biased	0.12
Sepsis flavimana Meigen, 1826	Zürich, Switzerland	female-biased	0.09
Sepsis lateralis Wiedemann, 1830	La Laguna, Spain	male-biased	-0.03
Sepsis neocynipsea (EU) Melander & Spuler, 1917	Sörenberg, Switzerland	female-biased	0.05
Sepsis neocynipsea (NA) Melander & Spuler, 1917	Montana, USA	male-biased	-0.04
Sepsis orthocnemis Frey, 1908	Zürich, Switzerland	female-biased	0.07
Sepsis punctum (EU) (Fabricius, 1794)	Zürich, Switzerland	male-biased	-0.07
Sepsis punctum (NA) (Fabricius, 1794)	Syracuse, USA	female-biased	0.03
Sepsis thoracica (Robineau-Desvoidy, 1830)	Zürich, Switzerland	male-biased	-0.08

sarily synonymous in other contexts, as the literature on allometry as well as condition dependence is complex (e.g. Pelabon et al. 2013, Hill 2011, Johnstone et al. 2009).

Black scavenger flies (Diptera: Sepsidae) are well suited for studying the relationship between sex-specific condition dependence and sexual dimorphism, as their morphology is highly plastic and traits vary strongly in their direction and degree of sexual dimorphism. Moreover, previous studies have demonstrated considerable variation in mating systems among taxa that goes hand in hand with sex-specific directional selection and directional variation in sexual size dimorphism (SSD) (Puniamoorthy et al. 2012a; Puniamoorthy et al. 2012b; Rohner et al. 2016). Generally, scramble competition for access to mates with direct and indirect female choice tends to be associated with female-biased SSD (e.g. in *Sepsis cynipsea*: Blanckenhorn et al. 2000), whereas male-biased SSD is found in species with pronounced male-male competition (e.g. *S. thoracica* or *S. punctum*: Busso & Blanckenhorn 2018a; Zerbe 1993).

Taking advantage of their well-resolved phylogeny, our ability to rear multiple species under controlled laboratory conditions, and their large interspecific variation in sexual dimorphism, we here test for and quantify the relationship between sex-specific condition dependence and sexual dimorphism among traits within and across closely related sepsid species. We consider both putatively sexually and naturally selected traits to expand the extent of dimorphism and compare the degree of condition dependence between sexual and non-sexual, "control" traits (Bonduriansky 2007a; Cotton et al. 2004b; Fairbairn 2005). We additionally investigate the effect of reversals in sexual size dimorphism, a close correlate of mating-system variation, on the relationship between sex-specific condition dependence and dimorphism. Our ultimate goal is to test for systematic relationships between sex-specific scaling relationships, indicative of condition dependence, and sexual dimorphism in sexual versus non-sexual traits across the sepsid phylogeny.

Materials & Methods

Sepsids are usually associated with decaying organic matter (e.g. vertebrate dung, rotting plant material) as breeding substrate (Ozerov 2006; Pont & Meier 2002). Because such habitats are typically ephemeral, variation in the amount of resources available for an individual's metabolism and development (i.e. condition) is large under natural conditions. Sepsid flies consequently exhibit strongly plastic responses to food quantity and quality, involving both development time and adult body size (adult dry weight of siblings can vary by a factor of 10: Zerbe 1993; see also Blanckenhorn 1999; Dmitriew & Blanckenhorn 2014; Rohner et al. 2018).

Most sepsid species also show pronounced sexual dimorphism in various traits other than overall body size, with its degree being highly trait-specific. In the genus *Sepsis* Fallén 1810, males typically show marked modifications of their fore femur (Pont & Meier 2002). These include spines, bristles and protrusions that are absent entirely in females (compare figs. 1a-c; also see Sepsidnet, the digital reference collection for Sepsidae: http://sepsidnet-rmbr.nus.edu.sg/; Ang et al. 2013). This strongly modified fore femur is used to hold on to the female's wing base during copulation and is therefore believed to be under sexual selection (Blanckenhorn et al. 2004; Eberhard 2001b), although its function is likely also stimulatory and thus complex (Eberhard 2001b, 2005; Ingram et al. 2008). Other appendages are also used during copulatory courtship, including tactile and visual stimulation of the female with the male mid legs (Puniamoorthy 2014; Puniamoorthy et al. 2009), or the transmission of chemical compounds originating from the osmeterium located on the hind tibiae onto the female wing (Eberhard 2001a). Yet, their functions often remain elusive (Araujo et al. 2014).

Whereas the male (but not female) legs are probably sexually selected in at least some sepsid species, other traits, like the thorax or wings, likely are not. Unless involved in courtship, insect wings are typically under stabilizing natural (i.e. viability) selection, such as in *Drosophila melanogaster* (Gilchrist et al. 2000). In *Sepsis*, both females and males almost permanently wave their wings, a behavior that is therefore not specific to the mating context

(Pont & Meier 2002). Similarly, the insect thorax, which primarily harbors the flight muscles, is also likely to be subject to stabilizing selection with no (or limited) function during courtship or mating.

Taxon sampling

To quantify sexual dimorphism and condition dependence, we experimentally reared nine closely related species of black scavenger flies (table 1). As populations of *Sepsis neocynipsea* and *S. punctum* evolved divergent mating systems and morphologies on different continents (EU: Europe, NA: North America; Puniamoorthy et al. 2012a,b; Rohner et al. 2016), we treated these populations as independent evolutionary lineages (hereafter referred to as taxa). All taxa were kept in large, outbred laboratory cultures (200-300 individuals) for several generations prior to any experimental procedure.

Condition dependence

Condition dependence can be estimated in several sensible ways (e.g. Hill & Farmer 2005, Cotton et al. 2004b, Bonduriansky et al. 2015). Comparing species with very different body sizes and levels of sexual size dimorphism, however, we could not use discrete environmental treatments because similar environments (e.g. food amounts) are unlikely to have identical effects across species. We therefore used a continuous environmental treatment to estimate sex- and trait-specific static allometries, which permit standardized comparisons across species and the sexes controlling for overall body size differences. Although this is no quantitative genetic study because we used food to manipulate phenotypes in population cages, by quantifying evolved sex differences across species in a standardized way, we are indirectly addressing the evolution of sex-specific genotype-by-environment effects in the broadest sense.

We first induced variation in condition by manipulating food availability during the larval stage. To this end, we collected eggs from outbred laboratory cultures by providing adult flies with a petri dish filled with cow dung. After one day, the petri dish was removed and the eggs laid were haphazardly distributed among plastic containers with varying amounts of homogenized cow dung (ranging from 10 individuals per 3g up to 10 individuals per 30g), and subsequently incubated at constant 18°C. For each species, we set up 10 to 30 replicates. Note that in contrast to flies reared with overabundant food, strong food limitation caused very high levels of larval mortality in all species, suggesting that we approximate the full phenotypic body size range given the temperature regime. Upon emergence and complete hardening, all individuals were frozen and stored in 70% ethanol. Once all individuals had emerged, we haphazardly selected 30 to 50 individuals per sex and species covering the full phenotypic range for morphological measurements.

The right fore-, mid-, and hind legs, as well as the right wing, were removed from the thorax and mounted on a glass slide using Euparal. We subsequently took pictures of all dissected appendages as well as the thorax (lateral view) using a Leica DFC490 camera mounted on a Leica MZ12 microscope. The lengths of the fore femur, fore tibia, mid tibia, hind tibia and the thorax were measured using digitized landmarks (derived from tpsDig: Rohlf 2009; fig. 1). We also estimated wing length and width as depicted in fig. 1d.

Fore femur morphology differs strongly among species, ranging from a sheer lack of any modification (e.g. *Sepsis duplicata*) to pronounced exaggeration (e.g. *S. lateralis*, *S. punctum*). As the apparent degree of exaggeration does not necessarily relate to femur length, we quantified investment in fore femur morphology by estimating its average width. We thus measured the total area of the fore femur (following Busso & Blanckenhorn 2018b) and divided it by its length.

Hind tibia length was among the traits loading most strongly on the first principal component (PC1; Table S1) and was always close to isometry when calculating (sex-specific) multivariate allometric slopes (Jolicoeur 1963; Figure S1); it was therefore used as our best estimate of overall body size (as in previous studies; e.g.: Blanckenhorn 2007; Martin & Hosken 2004; Rohner et al. 2018). To test for taxon and sex effects on overall body shape allometry, we first used MANCOVA fitting all focal traits as a function of log hind tibia length, sex, taxon and their interactions using type III Sums of Squares. Static trait- and sex-specific allometric slopes were calculated by regressing log trait

size against log hind tibia length in a reduced major axis regression (RMA). We tested for intra-specific associations of the degree of trait-specific condition dependence between the sexes using Pearson's product moment correlations. We further used the logarithm of the ratio between the male and female allometric RMA slopes as an index of sex-specific condition dependence (positive if males have steeper allometries).

Sexual dimorphism

As some taxa investigated here secondarily evolved male-biased sexual size dimorphism (Rohner et al. 2016), comparing absolute trait sizes between species does not necessarily reflect sex differences in the relative investment in a trait. To remove any variation due to overall body size, we first calculated the residual trait size derived from a regression of trait size against hind tibia length using all data (sexes were pooled). These residuals were z-scored and averaged by sex. The average difference between the sexes then represents our size-controlled index of *relative* sexual dimorphism. In what follows, we refer to this measure unless specifically discussing sexual *size* dimorphism (SSD).

Relationship between sex-specific condition dependence and sexual dimorphism

To test for a relationship between sex-specific condition dependence (i.e. sexual differences in allometric slopes) and relative sexual dimorphism among traits, we calculated Pearson's correlation coefficients for each species separately. To test for a general relationship independent of species and trait identity, we pooled all data and used a mixed model with species and trait as crossed random effects (using the *lme4* R-package: Bates et al. 2015).

Our comparative approach further allowed testing for the coevolution of sex-specific condition dependence and sexual dimorphism of each trait across the phylogeny. To this end, we used phylogenetic generalized least-squares (PGLS) models (using the R-package *caper*: Orme et al. 2013) with the average species- and sex-specific condition dependence as response and relative sexual dimorphism as the predictor variable. To account for the phylogenetic relationships among species, we used a cropped version of the phylogeny published by Zhao et al. (2013), setting all branch lengths to unity. The branch length transformation parameter λ was estimated using maximum likelihood.

Linking mating system variation to sex-specific scaling relationships

Animal species in which males are the larger sex, such as most mammals, typically show male-male competition for access to females, while in species with female-biased sexual size dimorphism males typically scramble for access to mates and females are choosier (Andersson 1994; Blanckenhorn 2005; Fairbairn 1997; Lüpold et al. 2015). Accordingly, the intensity of pre-copulatory sexual selection on male size correlates with the direction of sexual size dimorphism in sepsid flies (Puniamoorthy et al. 2012a,b; Rohner et al. 2016): in species or populations in which males are the larger sex, males show pronounced male-male competition or combat behavior that is also apparent under laboratory conditions (Busso & Blanckenhorn 2018a; Eberhard 1999, 2002; Rohner et al. 2016; Zerbe 1993). We therefore used the direction of dimorphism as a crude proxy for the mating system, i.e. the presence of pronounced male-male competitive behavior. (Note that using a continuous index of sexual dimorphism shows qualitatively similar results.) To test for an association between the mating system and body shape or scaling relationships, we compared sex-specific allometric slopes and relative sexual dimorphism of taxa with male- and female-biased dimorphism using PGLS.

Results

Sex-specific condition dependence

Overall body shape varied strongly with body size (MANCOVA; log hind-tibia length: $F_{7,838} = 19149.2$; $P < 0.001$; $\eta_p^2 = 0.99$), among taxa ($F_{70,6510} = 100.5$; $P < 0.001$; $\eta_p^2 = 0.48$), and between the sexes ($F_{7,838} = 746.2$; $P < 0.001$; $\eta_p^2 = 0.85$). Apart from these main effects, all interactions were statistically significant: that is, allometric relationships differed between taxa (taxon × log hind-tibia length: $F_{77,5908} = 9.6$; $P <$

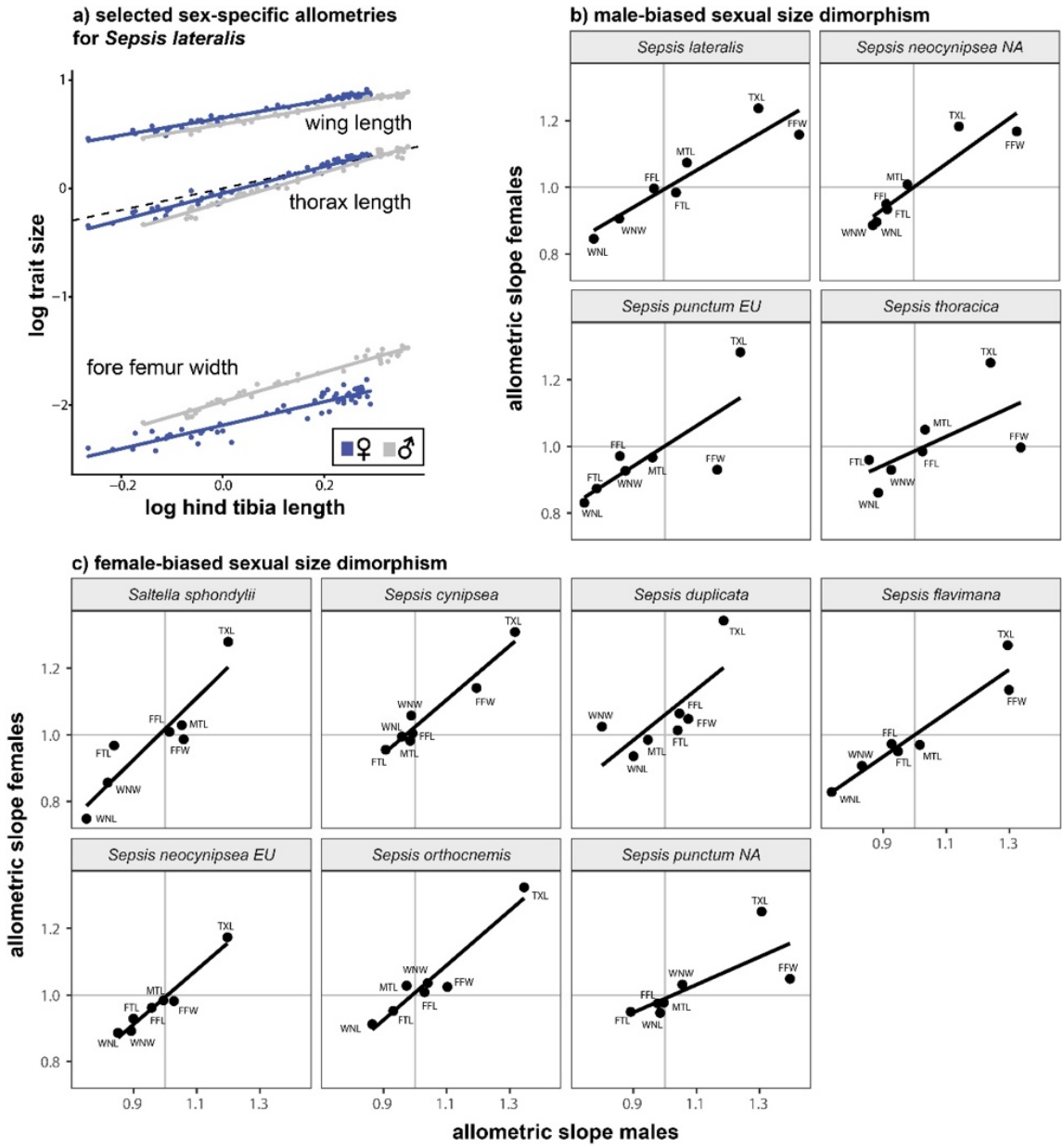

Figure 2: Static allometric (RMA) slopes (relative to hind tibia length) differ for various traits and somewhat between species, but generally correlate strongly between the sexes. Particularly thorax length is strongly hyperallometric in all taxa, while wing length and width are hypoallometric. Panel a) shows sex-specific allometries for wing length, thorax length and fore femur width as an example (isometry is indicated by a broken line). Panels b) and c) depict patterns in taxa with male- and female-biased SSD, respectively. (FFL: fore femur length; FFW: fore femur width; FTL: fore tibia length; MTL: mid tibia length; TXL: thorax length; WNL: wing length, WNW: wing width; see figure 1).

0.001; $\eta_p^2 = 0.17$) and the sexes (log hind-tibia length × sex: $F_{7,838} = 15.1$; $P < 0.001$; $\eta_p^2 = 0.14$). Sex-specific allometries and sex differences in overall body shape further differed between taxa (taxon × sex: $F_{70,5908} = 19.6$; $P < 0.001$; $\eta_p^2 = 0.18$; taxon × log hind-tibia length × sex : $F_{70,5908} = 2.4$; $P < 0.001$; $\eta_p^2 = 0.03$).

In both males and females, thorax length and fore femur width tended to be strongly hyperallometric (fig. 2, table S2), while wing length and wing width were hypoallometric (all relative to hind-tibia length; fig. 2, table S2). Allometric slopes were strongly correlated between the sexes (linear mixed model using trait identity and species as random effects: $F_{1,73.8} = 59.1$; $P < 0.001$; $r = 0.67$ [95%CI: 0.53, 0.76]; $P < 0.001$).

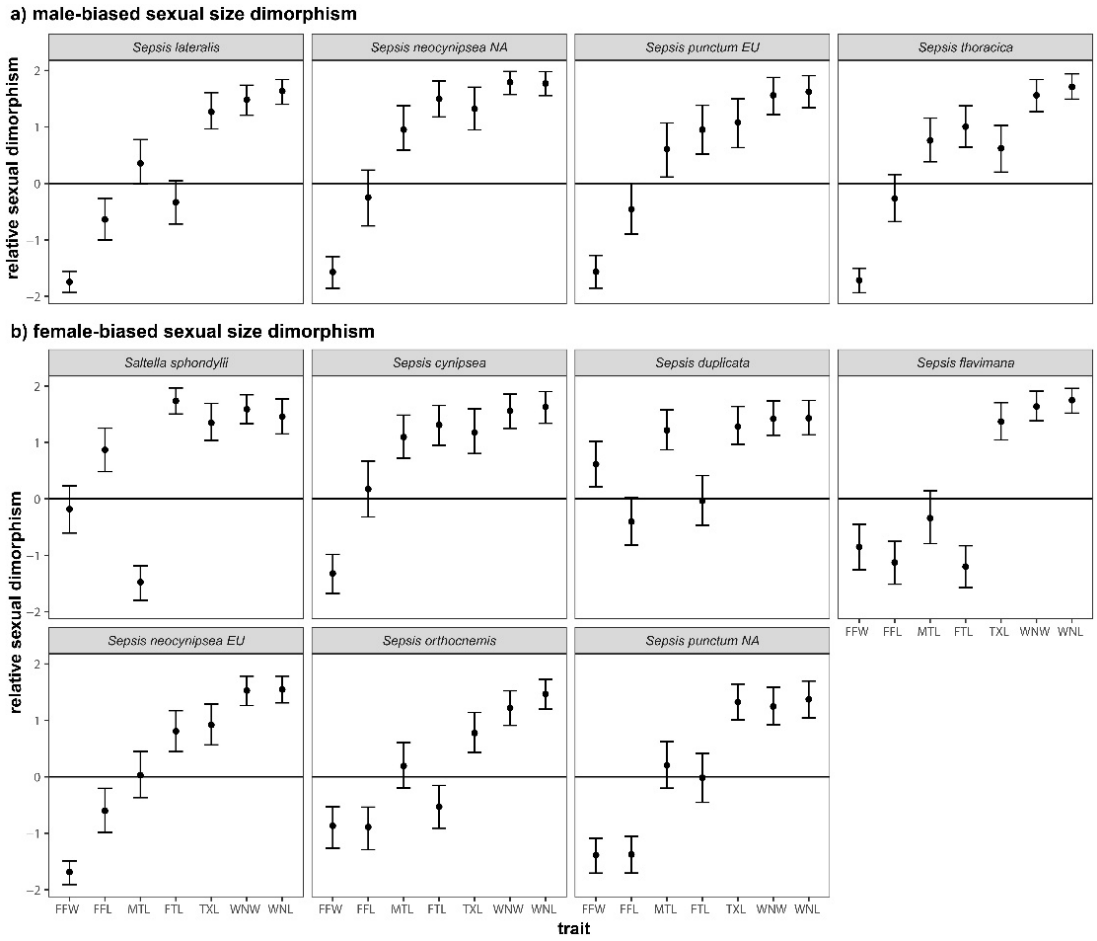

Figure 3: Relative sexual dimorphism (corrected for dimorphism in overall size, i.e. hind tibia length) varies strongly among traits within species, though patterns are rather consistent across species. Positive scores indicate bias towards males; negative scores female- based dimorphism. Error bars represent 95% bootstrap confidence limits. (FFW: fore femur width; FFL: fore femur length; MTL: mid tibia length; FTL: fore tibia length; TXL: thorax length; WNW: wing width; WNL: wing length; see figure 1).

Sexual dimorphism

Relative sexual dimorphism varied strongly among traits (fig. 3). Thorax length, wing width and length were always larger in females, whereas fore femur width and length were larger in males except for *Saltella sphondylii* and *Sepsis duplicata*, which both lack pronounced sex-specific modification of this body part (Pont & Meier 2002). Fore- and mid-tibia length showed more idiosyncratic patterns across species (see fig. 3).

Relationship between sex-specific condition dependence and sexual dimorphism

The extent of sexual dimorphism correlated positively with the sex difference in condition dependence (linear mixed model using trait and taxon as random effects: $X^2_{(1)} = 16.80$; $P < 0.001$; $r = 0.52$ [0.28, 0.68]). Hence, traits that are relatively larger in males also more strongly depend on condition in males (and *vice versa*). This relationship remained statistically significant when fore femur width, the most pronounced sexually dimorphic trait, was excluded from the analysis ($X^2_{(1)} = 5.43$; $P = 0.020$). Note that these relationships were not often significant when tested within species (table 2), but a strong overall pattern persisted (average $r = 0.66 \pm 0.04$ (SE)). Interestingly, this relationship among traits was stronger in taxa with pronounced male-male competition ($r = 0.75 \pm 0.02$ SE, $n = 4$) than in taxa with more female choice ($r = 0.60 \pm 0.05$ SE, $n = 7$) (PGLS: $F_{1,9} = 7.30$, $P = 0.024$).

We also found a positive relationship between sex-specific condition dependence and sexual dimorphism for fore femur width, fore tibia length, and the length of the mid tibia

across species (fig. 4). Taxa that evolved increased sexual dimorphism in these traits hence also evolved increased differences in condition dependence between the sexes. The remaining traits showed much weaker and non-significant patterns (fig. 4).

Linking mating system variation to sex-specific scaling relationships

Taxa with male-biased SSD (and more male-male competition) had broader and more condition-dependent (i.e. more hyperallometric) male fore femora (relative size; PGLS: $F_{1,9}$ = 9.16, P = 0.014, fig. 5a; static allometric coefficient: PGLS: $F_{1,9}$ = 9.65, P = 0.013, fig. 5b). All other traits did not systematically present such differences (not shown).

Discussion

We here demonstrated that sex-specific condition dependence and sexual dimorphism consistently co-vary among traits within 11 sepsid taxa. Using a comparative approach, we further showed that this relationship extends to the interspecific level. Species with higher degrees of sexual dimorphism exhibit greater sex differences in their condition dependence for sexual, but not necessarily for non-sexual traits: the overall correlation based on all traits and taxa was r = 0.52 [0.28, 0.68]. As a whole, our results support the prediction that sexually antagonistic directional selection not only mediates the evolution of sexual dimorphisms but also the establishment of sex differences in condition dependence, both within and across species. This relationship is stronger in taxa that secondarily evolved male-biased SSD with pronounced male-male competition (the derived state in this group of flies), likely driven by stronger sexual selection on overall body size and/or fore femur width in males (Busso & Blanckenhorn 2018a; Dmitriew & Blanckenhorn 2012; Puniamoorthy et al. 2012a,b; Rohner et al. 2016). In what follows, we elaborate on the causes and consequences of this co-evolution of sexual dimorphism and condition dependence, and discuss how mating system variation may influence the evolution of scaling relationships.

Ultimately, different male and female phenotypes are thought to evolve due to sexually divergent fitness optima, resulting from variation in the strength, shape and direction of natural, sexual and viability selection between the sexes (Blanckenhorn 2005; Fairbairn 2013; Fairbairn et al. 2007; Hedrick & Temeles 1989; Shine 1989), although the specific role of ecology in shaping sexual dimorphisms remains contentious, particularly in insects without parental provisioning (Blanckenhorn 2005). However, even under consistent and sexually antagonistic directional selection, the evolution of sexual dimorphism in any trait must be hampered by genetic correlations between sexes (Lande 1980). As conspecific males and

Table 2: Among-trait correlations between sex-specific condition dependence and sexual dimorphism for the eleven taxa investigated, here grouped by the direction of sexual size dimorphism (male or female larger). Traits that are relatively larger in males thus also tend to exhibit a corresponding increase in their allometric slope compared to females, and vice versa (global average correlation: 0.66 ± 0.04 (SE)).

Sexual size dimorphism	Species	t	df	r [95% CI]
female larger	*Saltella sphondylii*	2.01	5	0.67[-0.11, 0.8]
	Sepsis cynipsea	2.92	5	0.79[0.07, 0.87]
	Sepsis duplicata	1.71	5	0.61[-0.18, 0.77]
	Sepsis flavimana	1.47	5	0.55[-0.23, 0.75]
	Sepsis neocynipsea (EU)	2.04	5	0.67[-0.11, 0.8]
	Sepsis orthocnemis	1.35	5	0.52[-0.25, 0.73]
	Sepsis punctum (NA)	0.99	5	0.40[-0.33, 0.69]
				average r: 0.60 (± 0.05 SE)
male larger	*Sepsis lateralis*	2.63	5	0.76[0.01, 0.85]
	Sepsis neocynipsea (NA)	2.52	5	0.75[-0.01, 0.84]
	Sepsis punctum (EU)	2.19	5	0.70[-0.08, 0.82]
	Sepsis thoracica	3.03	5	0.80[0.09, 0.87]
				average r: 0.75 (± 0.02 SE)

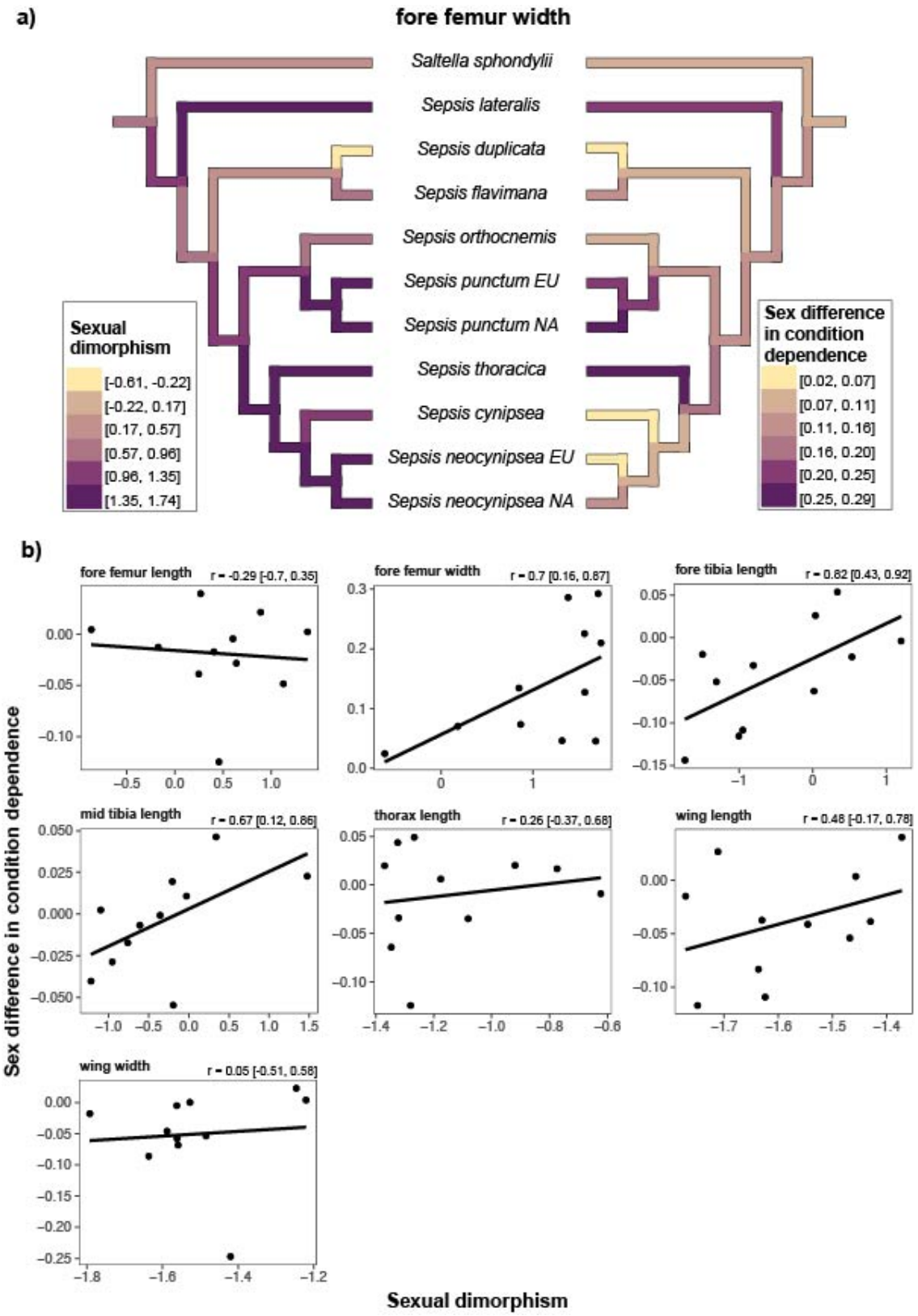

Figure 4: Sex-specific condition dependence as a function of sexual dimorphism among species. Figure (a) depicts ancestral character reconstruction for fore femur width as an example (squared-change parsimony; for illustration only), while the remaining plots (b) show raw, uncorrected values for all seven traits. Phylogenetically corrected correlation coefficients with corresponding 95% confidence limits are given.

females usually share most of their genome, including genes controlling the basic parameters of growth and development (Fairbairn 1997), such between-sex correlations can be strong and must be overcome in order to reduce or resolve intersexual (onto)genetic conflict

(Badyaev 2002; Rice & Chippindale 2001). In theory, sexual conflicts can be overcome if condition dependence can evolve in a sex-specific manner (Badyaev 2002). At minimum requiring the evolution of a sex-linked locus causing condition-dependent expression in merely one sex, this would permit optimal investment in secondary sexual traits without displacing the opposite sex from its fitness optimum - a form of sex-limited epistasis. While resolving sexual conflict, this would also establish a common genetic and developmental basis for sexual dimorphisms and condition dependence.

Such mechanisms are evident in several previously studied species, for instance in the neriid fly *T. angusticollis*. Bonduriansky (2007a) showed that condition dependence and sexual dimorphism are tightly correlated among traits in this species, suggesting that most (if not all) sexual dimorphism in various traits is caused by differential sex-specific condition dependence, such that empirically the two concepts are difficult to distinguish. Similarly, Ledon-Rettig & Moczek (2016) found that condition dependence and sexual dimorphism share genetic underpinnings at the transcriptome level, also suggesting a common developmental basis. Corroborating these results and extending them to the comparative level, we here found that although allometric slopes (signifying condition dependence) always correlate strongly between the sexes (fig. 2), more dimorphic traits also feature larger differences in their sex-specific condition dependence across eleven closely related taxa (table 2). This result documents a strong and consistent pattern in sepsid flies. Measuring seven traits in eleven species allowed us to estimate that sex-specific condition dependence explained merely about 30% of the variance in sexual dimorphism across species and traits. Compared to *T. angusticollis*, in which 90% of the variance in sexual dimorphism was explained by sex-specific condition dependence (Bonduriansky 2007a), sexual dimorphism in overall shape of sepsid flies thus seems far less condition-dependent. Although direct comparisons between the neriids and sepsids are hampered by different traits and methodologies used, the markedly different variance components reported nonetheless suggest varying degrees of interdependence between condition dependence and sexual dimorphism across taxa (and traits), implying that this dependency can therefore evolve.

Such interspecific variation is also evident among the sepsids examined here in that species with male-biased SSD, generally featuring more male-male competition, showed a significantly tighter relationship between trait dimorphism and sex-specific condition dependence ($r = 0.75$) than species with female-biased SSD ($r = 0.60$). On the one hand, such directional reversals of size dimorphism might be rather recent and, in response, sex-specific condition dependence (i.e. plasticity) may be more

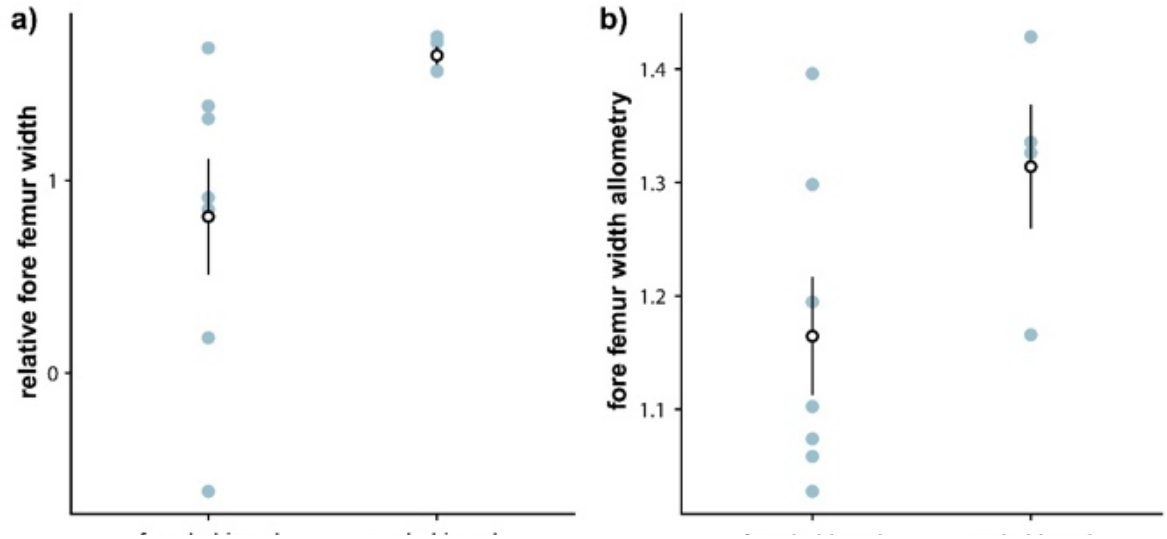

Figure 5: Taxa with male-biased sexual size dimorphism (the derived character state; cf. Rohner et al. 2016) show (a) increased investment in relative fore femur width, and (b) steeper allometric slopes (i.e. condition dependence) for this trait. Plots show raw, uncorrected values. Phylogenetically corrected statistics are given in the text.

likely to evolve than functional genetic sex differences, particularly if plasticity can better alleviate any constraints imposed by strong genetic correlations between sexes (as argued above; Lande 1980). In the long run, however, the evolution of (fixed) sex linkage might subsequently weaken such sex-specific condition dependence, as appears to be the case in the taxa with the ancestral female-biased SSD.

On the other hand, the difference between taxa exhibiting either male- or female-biased dimorphism may well relate to variation in the selective regime imposed on males. If sexual selection leads to the evolution of larger males, secondary sexual traits might acquire novel functions or, alternatively, their ancestral function may persist and be further strengthened during combat or courtship through positive selection. That is, sexual selection may promote further evolutionary amplification of the hyperallometric male slope (Bonduriansky 2007c; Gould 1966), thus strengthening the relationship between sex-specific condition dependence and sexual dimorphism of particular secondary sexual and other morphologically integrated traits. Although mostly anecdotal, observations in both the laboratory and the field suggest heightened degrees of male-male competition for access to females in those sepsids that secondarily evolved male-biased SSD. For instance, whereas males of species with female-biased SSD use their fore legs primarily to interact with the female during their elaborate pre-copulatory courtship displays, males of species with male-biased SSD use their modified fore femora to fend off nearby competitors (Eberhard 1999, 2002) or attempt to vigorously dislodge males that are already mounted on females (particularly evident in North American vs. European *S. punctum*: Zerbe 1993, Puniamoorthy et al. 2012a). In taxa with male-biased SSD, the armored foreleg therefore gained additional functions in male-male competition. Accelerating selection may thus have tightened the relationship between dimorphism and condition dependence (and possibly other morphologically integrated traits) in taxa with male-biased SSD. Future research will be necessary to experimentally assess the underlying drivers of this variation.

We here comprehensively extended empirical micro-evolutionary evidence to the macro-evolutionary level, a prime goal of evolutionary ecology. Aligning with the overall sex-specific body size plasticity, which generally correlates with the degree and direction of SSD across insects (Rohner et al. 2018; Stillwell et al. 2010; Teder & Tammaru 2005), we here found trait-specific support for this pattern across species. We uncovered significant correlations between (relative) sexual dimorphism and sex differences in condition dependence only for the investigated fore- and mid-leg traits (fig. 4): taxa that evolved relatively broader fore femora and longer fore tibiae in males showed heightened condition dependence in males compared to females. The same applies to the length of the mid tibiae. Such relationships are expected if the costs and benefits of increased trait investment in males are shared among species. Then, increased selection on trait dimorphism drives the evolution of condition dependence because large individuals benefit more from trait production and/or maintenance. This scenario apparently only applies to the fore legs, which are sexually selected in some species, but not in others, and indeed appear to be costly (as indicated by more hyperallometric growth compared to females: Blanckenhorn et al. 2000; Busso & Blanckenhorn 2018a; Dmitriew & Blanckenhorn 2012, 2014; Parker 1972; Puniamoorthy et al. 2012a,b; Zerbe 1993). The mid tibia is frequently observed to be used in the transmission of chemical compounds or the stimulation of the female during copulation and thus likely also appears to be under sexual selection in males (Araujo et al. 2014; Eberhard 2001a; Puniamoorthy 2014; Puniamoorthy et al. 2009). In contrast, patterns were weak and non-significant in all those traits that do not seem to play a major role in the sexual context (e.g., thorax, wing). This observed heterogeneity among traits may thus relate to varying levels of sexual selection across species, to the extent that only traits under consistent directional selection may drive the pattern of coevolution observed. This contrasts with cases where dimorphism is associated with selection driven by ecological niche differentiation between sexes (i.e. ecological sexual dimorphism: Temeles et al. 2000, Shine 1989, 1991). When the sexes represent different ecotypes, we expect stabilizing, and not directional, selection acting on the same trait in each sex, and condition dependence should not play a major role if dimorphism is truly ecological.

Conclusions

Across eleven closely related sepsids with varying mating systems, we found support for a relationship between sex differences in condition dependence and sexual trait dimorphisms among traits. Although these correlations were overall weaker than in some previously studied insects (Bonduriansky 2007a,b,c), this suggests a common developmental basis. Our findings reiterate that sex-specific condition dependence and sexual dimorphism are not necessarily independent biological properties, as the latter may arise through the former at least to some extent. We further found this relationship to co-evolve for traits that are sexually selected in males of some but not other species (fore- and mid-leg morphology), particularly in species that secondarily evolved male-biased sexual size dimorphism (*S. thoracica, European S. punctum, S. lateralis, North American S. neocynipsea*). Other traits assumed to be primarily under natural selection (thorax and wings) showed no such association. Although we here performed a phenotypic and not a quantitative genetic study, we systematically compared sex differences in trait plasticity of closely related species that likely share genetic mechanisms. In manipulating food availability, we thus estimated mere environmental (V_e) as well as corresponding genotype-by-environment effects (V_{gxe}), which presumably evolved due to similar selection pressures in the past. Which and how many genes are involved in regulating such sex-specific trait expression remains to be documented.

Whereas the evidence on the relationship between condition dependence and trait dimorphism presented here and elsewhere (Bonduriansky 2007a-c; Cotton et al.2004a,b; Wilkinson & Taper 1999) refers mostly to insects, we suspect it to be a general pattern in organisms where sexually antagonisitic directional sexual selection drives dimorphism. After all, the underlying proximate causes of sexual size dimorphism in terms of growth and developmental mechanisms, both of which are strongly dependent on environmental conditions, are well established in many other vertebrate and invertebrate taxa (Blanckenhorn et al. 2007; Bro-Jørgensen 2007; Jarman 1983; Leigh 1992; Teather & Weatherhead 1994). Nevertheless, the causes and consequences of this phenomenon warrant further mechanistic scrutiny, particularly at the physiological and genetic levels (Emlen et al. 2012; Shingleton & Frankino 2018; Tang et al. 2011).

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RESEARCH PAPER

WILEY

Critical weight mediates sex-specific body size plasticity and sexual dimorphism in the yellow dung fly *Scathophaga stercoraria* (Diptera: Scathophagidae)

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Ultimate factors driving insect body size are rather well understood, while—apart from a few model species—the underlying physiological and developmental mechanisms received less attention. We investigate the physiological basis of adaptive size variation in the yellow dung fly *Scathophaga stercoraria*, which shows pronounced male-biased sexual size dimorphism and strong body size plasticity. We estimate variation of a major physiological threshold, the critical weight, which is the mass at which a larva initiates pupariation. Critical weight was associated with sexual size dimorphism and sex-specific plasticity, and is thus a likely target of selection on adult size. Detailed larval growth trajectories derived from individuals raised at two food and temperature treatments further reveal that sex-specific size plasticity is mediated by faster initial growth of males that later becomes reduced by higher male weight loss during the wandering stage. We further demonstrate that integral growth rates, which are typically calculated as simple ratios of egg-to-adult development time and adult weight, do not necessarily well reflect variation in instantaneous growth rates. We illustrate the importance of detailed assessments of ontogenetic growth trajectories for the understanding of adaptive size variation and discuss the mechanistic basis of size determination in shaping sex-specific phenotypic plasticity.

1 | INTRODUCTION

Body size varies dramatically between and within species and contributes greatly to the phenotypic diversity observed across the tree of life. Evolutionary biologists have heavily scrutinized the ultimate drivers of body size variation, revealing an often tight relation of body size to fitness (reviewed in Andersson, 1994; Blanckenhorn, 2000; Chown & Gaston, 2010). Large individuals often leave more offspring, are more successful in acquiring mates, and survive better (Blanckenhorn, 2000; Clutton-Brock, 1988; Honěk, 1993; Shine, 1989). While the ultimate causes of body size variation thus are rather well understood, the proximate mechanisms determining body size remain poorly investigated apart from a few model species, despite their importance for understanding the evolutionary process (Badyaev, 2002; Chown & Gaston, 2010; Stillwell, Blanckenhorn, Teder, Davidowitz, & Fox, 2010).

In insects, intraspecific body size variation is striking and plastic responses to the environment and sexual dimorphism (SSD) can be extreme, but the underlying physiological and developmental causes have received surprisingly little attention (Badyaev, 2002; Blanckenhorn, 2000; Stillwell et al., 2010). As insect growth is determinate, all structural growth is restricted to the immature stages. It follows that adult size is a function of propagule (i.e., initial) size, growth rate, and the time over which juveniles grow. Nevertheless, body size may only indirectly depend on the rate or duration of growth. At

least as important are the mechanisms that terminate growth at a particular size or point in time (Nijhout, 2003; Nijhout & Davidowitz, 2009; Davidowitz, 2016; see below). Thus, when studying body size variation, be it caused by environmental or genetic processes, the consideration of the complexity of larval growth, as well as mechanisms of size determination, are crucial for the understanding of body size evolution.

Larval growth and the determination of adult size are best understood in *Manduca sexta* and *Drosophila melanogaster* (Mirth & Riddiford, 2007; Nijhout, 2003; Nijhout et al., 2014). In *Drosophila*, after hatching from the egg, larvae start to feed and grow nearly exponentially, leading the larva to moult and expand its cuticle. In their third instar, larvae reach a threshold size called the critical weight (or critical size in the *Drosophila* literature). The attainment of this threshold size is mediated by insulin/insulin-like growth factor signaling acting on the prothoracic gland, which in turn is thought to incite well-described endocrine signaling cascades that lead to the cessation of growth and the onset of pupariation to commence metamorphosis (reviewed by Mirth & Riddiford, 2007; Mirth et al., 2014; Nijhout et al., 2014; Shingleton, 2011). Once the larva has stopped feeding, it enters the wandering stage, during which it empties its gut and prepares for metamorphosis while actively looking for a suitable location for pupariation. It follows that variation in critical weight, marking the start of hormonal interactions leading to metamorphosis, will strongly influence the size of the adult insect. However, since larvae continue to grow until ecdysteroids are secreted, environmental plasticity or sex-specific variation in the terminal growth period (TGP) and the rate of growth during TGP can influence size as well. Variation in sex-specific size plasticity or adult sexual size dimorphism can thus arise from variation in critical weights, differences in the amount of growth during the TGP (which is affected by growth rate and duration), as well as disproportioned weight loss during the subsequent development. Critical weight variation has been shown to affect sexual size dimorphism (Testa, Ghosh, & Shingleton, 2013) and underlies the temperature-size-rule in *D. melanogaster* (Ghosh, Testa, & Shingleton, 2013). In *M. sexta,* in contrast, critical weight is unaffected by temperature (Davidowitz, D'Amico, & Nijhout, 2003; Stillwell & Davidowitz 2010) but nevertheless influences SSD (e.g., Stillwell, Daws, & Davidowitz, 2014). The extent to which these mechanisms apply to other insects is not yet fully clear (Parker & Johnston, 2006). It seems likely, however, that critical weight (or a similar mechanism) is a key player in body size regulation of all holometabolous insects (Callier & Nijhout, 2011; Stieper, Kupershtok, Driscoll, & Shingleton, 2008), although growth variation after the attainment of critical weight could account for adult size variation (e.g., SSD) as well.

The complexity of the larval growth just described is often neglected in organismic biology. This is not a matter of ignorance but rather a necessity given the efforts required in estimating detailed growth trajectories, especially in comparative and field studies, as well as a consequence of the necessary reductionism when studying life-history evolution (Davidowitz, 2016). Larval growth rate is then often approximated as the ratio of adult size and egg-to-adult development time, thus assuming linear growth. While these estimates are convenient and often the only available data, insect larvae neither grow continuously nor in a linear fashion. Linear growth rates and overall egg-to-adult development times are therefore compound traits that integrate the nonlinear, interactive nature of larval growth, and often the biological meaning ascribed to these measures remains obscure (Tammaru, Esperk, Ivanov, & Teder, 2010). To interpret such growth rate estimates (in the following referred to as "integral growth rates"; Figure 1) in a biologically meaningful way, their comparison to detailed larval growth trajectories is helpful, if not essential.

Here, we study larval growth and physiology in the yellow dung fly *Scathophaga stercoraria* (Linnaeus, 1758) (Diptera: Scathophagidae). This large coprophilous fly (7–13 mm in length; Blanckenhorn, Pemberton, Bussière, Roembke, & Floate, 2010) shows strong body size variation with male-biased sexual size dimorphism (Simmons & Ward, 1991), the adaptive nature of which has been well scrutinized. Body size varies genetically across latitude (Blanckenhorn & Demont, 2004) and (somewhat) altitude (Blanckenhorn, 1997). Plastic responses to habitat depletion (by a "bail-out" response *sensu* Tobler & Nijhout 2010), which is common because dung is an ephemeral habitat, as well as to seasonality and temperature are strong (Blanckenhorn, 1998, 1999, 2009). However, the underlying physiological and developmental mechanisms, the potential targets of selection, have received much less scrutiny (but see Blanckenhorn & Henseler 2005; Blanckenhorn & Llaurens, 2005).

By estimating detailed, individual growth trajectories under different environmental conditions we here aim to reveal the proximate causes underlying sex-specific body size plasticity in the yellow dung fly. We estimate sex-specific critical weight, as opposed to the commonly investigated minimum viable weight, and expect this major size determinant to cause adult body size variation. Additionally, we compare simple integral growth rates to the actual linearized weight increment with age during the initial, quasi-exponential growth phase (=quasi-instantaneous growth rates) to investigate whether the former estimates introduce systematic biases. We conclude by discussing the implications of growth and size determination for the evolution of body size plasticity and sexual size dimorphism.

2 | MATERIALS AND METHODS

Adult *S. stercoraria* were captured on a cattle pasture in Zurich, Switzerland and used to establish an outbred

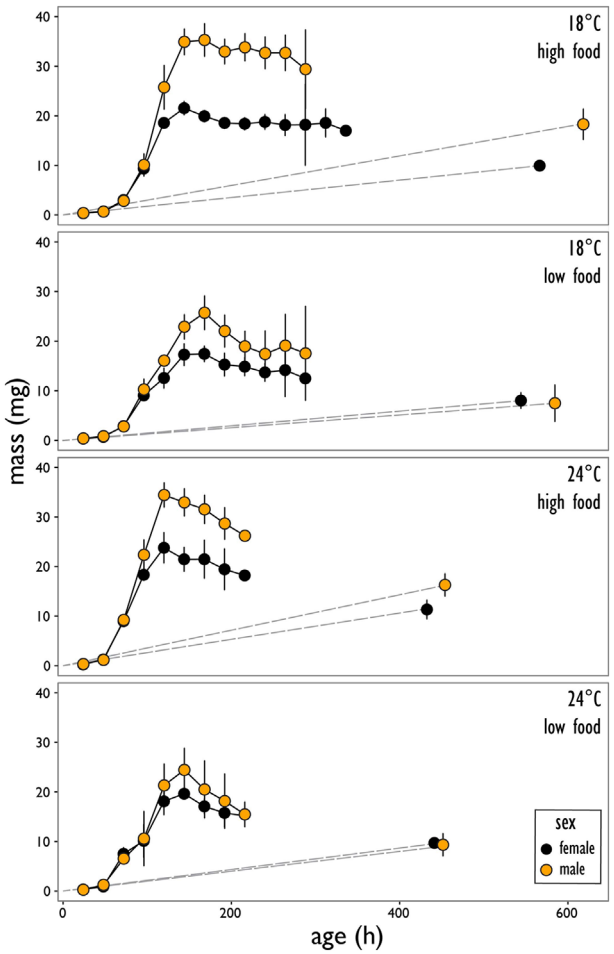

FIGURE 1 Sex-specific larval growth trajectories in different environments characterized by variation in dung quantity and temperature. Points (± 95%CI) represent mean mass of individually-weighed larvae at a given age, starting with newly hatched first instar larvae. Sex-specific mean adult mass is indicated at the mean age at eclosion (isolated points to the right). During early larval development (first ~4 days), larval mass increases nearly exponentially. Eventually, larval growth ceases and individuals enter the wandering stage during which weight is lost. Males (indicated in orange) grow faster but reach their peak weight at the same time as females (black dots). Sexual size dimorphism is always present at the larval peak weight but adults are monomorphic under food limitation due to a higher weight loss of males. Broken gray lines indicate integral growth rates derived from adult mass and egg-to-adult development times, illustrating the conceptual difference between instantaneous and integral estimates of growth rate estimates

laboratory culture under standard maintenance procedures (Blanckenhorn et al., 2010).

2.1 | Individual larval growth trajectories

To estimate detailed larval growth trajectories, we followed individual flies throughout their immature stages. Eggs were collected from the laboratory culture and singly placed onto the surface of a small rectangular dish (22×22 mm^2) filled with standardized, previously frozen cow dung. To prevent desiccation, the bottom of each dish was filled with a shallow layer of agar (3%). Larvae hatched within 24 hr and were recovered from the opaque substrate with a spatula, rinsed with tap water and dried on filter paper. Clean and dried larvae were then weighed twice to the nearest 0.01 mg and placed back into the same dish. The amount of dung supplied at the start of the experiment therefore, represented the total amount available to a larva to complete its development. The weighing procedure was repeated every 24 hr until larvae underwent pupariation. (Pre)pupae were weighed and checked for adult eclosion every other day. Upon eclosion, adults were killed by freezing, sexed, and weighed. The sex of individuals that died prior to adult eclosion or during the early pupal stages could not be assessed, so these individuals were removed from the data set. To assess plastic responses to environmental variation we used a two-factor design, crossing high and low temperatures (24 vs. 18°C) with unlimited and limited amounts of food (2 vs. 0.7 g of dung per individual). Dung amounts were chosen based on previous findings and personal experience (pers. obs. WUB). Under food limited conditions, young larvae are still able to dwell in and feed on dung in a regular manner. At some point however, nutrients deplete and larvae rummage through an empty cellulose matrix, vainly searching for food.

To assess sex-specific larval growth we measured several parameters. To quantify the speed of growth during the quasi-exponential period, we regressed cube-root transformed weight against age, and calculated the increase in linearized weight with time (slope of the regression in [mg$^{1/3}$/h]) for each individual separately. Only data gathered during the time in which larvae grow nearly exponentially was included. Because the relationship between cube-root transformed mass and growing time was approximately linear, the slope of this relationship could be used as an estimate of instantaneous growth rate. To test for differences in instantaneous growth rates between groups, we used a mixed linear model of mass$^{1/3}$ as a function of age, sex, food quantity, and temperature as fixed effects, their interactions, and the identity of each individual larva as random effect (cf., Blanckenhorn, 1999; Teuschl, Reim, & Blanckenhorn, 2007). In this model, the coefficient of the age term was used to quantify growth rate. Non-significant interaction terms were removed. To ensure that growth was indeed exponential, we here also only analyzed data for the first 96 hr (which represents approximately 2/3rds of the growth period) during which growth followed an exponential trend, irrespective of treatment or sex. To compare instantaneous growth rates to estimates of integral growth (= individual adult mass$^{1/3}$ divided by egg-to-adult development time), we used both estimates simultaneously as dependent variables in a bivariate analysis with the type of growth rate (instantaneous or

integral), sex, food quantity and temperature as explanatory factors and individual identity as random effect.

We further analyzed larval peak mass (maximum weight of the larva), the subsequent weight loss during the wandering phase (difference between larval peak mass and pupal mass), the mass at pupariation, as well as, adult mass using type III ANOVAs with sex, food quantity, temperature, and their interactions as explanatory variables. Measurements of mass were always cube root transformed, and non-significant interactions were removed. We also analyzed the duration of the exponential growth period (estimated graphically from the individual growth trajectory), the age at which growth stopped (age at which a larva reaches its peak mass), the duration of the wandering stage (difference between the age at larval peak mass and age at pupariation), and the ages at pupariation and adult eclosion using analogous type III ANOVAs.

2.2 | Critical weight

In *M. sexta* the critical weight is defined by the minimum mass of a larva at which starvation does not further prolong pupation and is usually estimated by comparing the time to metamorphosis of starved and fed larvae of different weight classes (Nijhout & Williams, 1974). The effect of starvation on the time to pupariation (TTP) is however not universal. Especially in insects inhabiting ephemeral habitats, metamorphosis is induced prematurely under starvation (Blanckenhorn, 1998, 1999; Shafiei, Moczek, & Nijhout, 2001; Stieper et al., 2008; Teder, Vellau, & Tammaru, 2014). These alternative life history strategies therefore, require alternative procedures to identify the weight at which a larva initiates pupariation. In *D. melanogaster*, an alternative "break-point" approach is commonly applied: following the prediction that critical weight should alter the relationship between mass at starvation and the time to pupariation (TTP), plotting the latter two against each other should result in a segmented relationship with a pronounced break-point at the critical weight (due to a change in reaction). Larvae that have not yet reached their critical weight are expected to continue their larval development, while larvae that passed their critical weight should be unaffected or show a so-called "bail-out" response (Tobler & Nijhout, 2010). Crucially, critical weight is different from the minimum viable weight (Davidowitz et al., 2003; Stieper et al., 2008), which is more often reported and sometimes used as a proxy for the critical weight; however, the minimum viable weight refers to the minimum amount of resources necessary to reach the next life stage (Mirth & Riddiford, 2007) and is not directly related to the induction of metamorphosis per se.

Since *S. stercoraria* shortens its development time when food is limited (Blanckenhorn, 1998, 1999), we here apply the break-point method. Analogous to the aforementioned rearing protocol, individual larvae were placed into small dishes with either limited or unlimited cow dung in a 18°C climate chamber. Over the course of several days, haphazardly chosen 3rd instar larvae (according to the cephaloskeleton morphology: Ferrar, 1987) were removed from their dish, weighed and placed individually into an empty dish equipped only with a thin layer of agar to prevent desiccation. Larvae that underwent such a starvation treatment were monitored every 12 hr to record the timing of either death or pupariation. If larvae successfully pupariated, their time to pupariation (TTP) was calculated as the time between the start of starvation and pupariation. Of the 1,150 larvae starved, only 700 made it to the adult or late pupal stage and could be sexed; data on all other individuals were discarded.

We plotted the time to pupariation (TTP) of each individual larva against its mass at starvation (Figure 2). If larvae have not yet passed their critical weight, pupariation has not yet been induced and larvae may try to forage more in order to reach their critical weight. This prolongs their TTP. Only after a certain time lag will these larvae eventually pupariate (possibly adjusting their target size to the new environment (i.e., modifying their critical weight)). In contrast, the TTP of larvae that have passed their critical weight and already induced metamorphosis, should not be affected by starvation, as JH is already being depleted and ecdysone secretion will inevitably kick in. The reaction of a larva in terms of TTP should thus vary according to its weight at starvation. Prior to reaching their critical weight, larvae are expected to prolong their TTP, which should not happen thereafter. Plotting TTP against larval mass at starvation resulted in an angular relationship with a break point, suggesting the existence of a *Drosophila*-like critical weight. We then fitted a segmented regression to this relationship (using the R-package *segmented*: Muggeo (2008) which also supplies confidence intervals), the break point of which indicates the critical weight (the weight at which the reaction to starvation changes). This was done separately for males and females in both limited and unlimited food conditions (Figure 2).

3 | RESULTS

3.1 | Plastic responses to temperature and food manipulation

Larvae raised at 24°C eclosed earlier than those raised at 18°C ($F_{1,61} = 141.38$, $p < 0.001$) but did not significantly differ in their adult mass ($F_{1,63} = 2.35$, $p = 0.130$). In the high temperature treatment, larvae had higher instantaneous growth rates (GLM with larval mass as dependent variable: age*temperature: $F_{1,196} = 64.78$, $p < 0.001$), but the duration of the exponential growth phase was shorter (main effect:

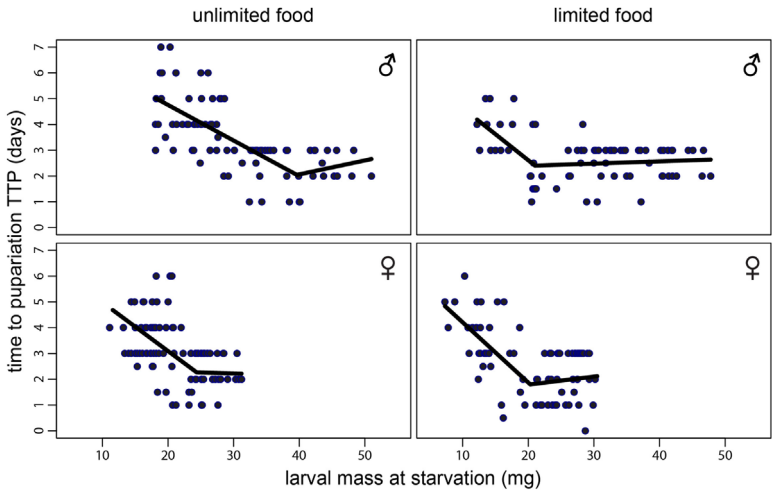

FIGURE 2 Plotting the time to pupariation (TTP) upon starvation against the mass at which larvae were starved results in an angular relationship. This suggests that larvae change their reaction to starvation depending on their size. The peak-points of the segmented regressions thus indicate the critical weights. These vary between sexes and food quantity treatments, suggesting a role of critical weights in shaping sex-specific plasticity (see also Figure 3)

$F_{1,60} = 106.38$, $p < 0.001$). Consequently, 24°C larvae reached their peak weight sooner (age at peak mass, main effect: $F_{,1,62} = 13.53$, $p < 0.001$, Figure 1) and showed a shorter wandering stage (main effect: $F_{1,60} = 42.32$, $p < 0.001$), thus pupariating earlier (main effect: $F_{1,60} = 105.00$, $p < 0.001$, Figure 1), and the duration of their pupal stage was shorter than that of larvae raised at 18°C (main effect: $F_{1,61} = 241.45$, $p < 0.001$, Figure 1).

Food quantity had somewhat similar effects: instantaneous growth rates were higher under food limitation (GLM with larval mass as dependent variable: age*food limitation: $F_{1,196} = 13.59$, $p < 0.001$) and larvae terminated their exponential growth phase earlier ($F_{1,60} = 5.21$, $p = 0.026$). Food limitation had no effect on the timing of growth cessation (age at larval peak mass), but it did affect larval peak mass ($F_{1,61} = 27.90$, $p < 0.001$). The wandering stage of food limited larvae was shorter ($F_{1,60} = 5.67$, $p = 0.020$; corrected for body size) and, as a result, they pupariated earlier. Weight loss during the wandering stage was independent of food quantity, but the lower peak weight of food-limited larvae resulted in lighter pupae ($F_{1,61} = 7.55$, $p = 0.008$). Finally, the duration of the pupal stage did not depend on food limitation but larger individuals took longer to eclose (duration of pupal stage: $F_{1,61} = 28.74$, $p < 0.001$). All AN(C)OVA tables as well as the results of the GLMs are presented in full in the supplementary Tables S1 and S2.

3.2 | Ontogeny of sexual size dimorphism

Overall, the larger males had a higher instantaneous growth rate than females (GLM with larval mass as dependent variable; age*sex interaction: $F_{1,64} = 17.05$, $p < 0.001$), and they also grew for longer in an exponential fashion (sex effect: $F_{1,60} = 20.71$, $p < 0.001$; sex*temperature interaction:

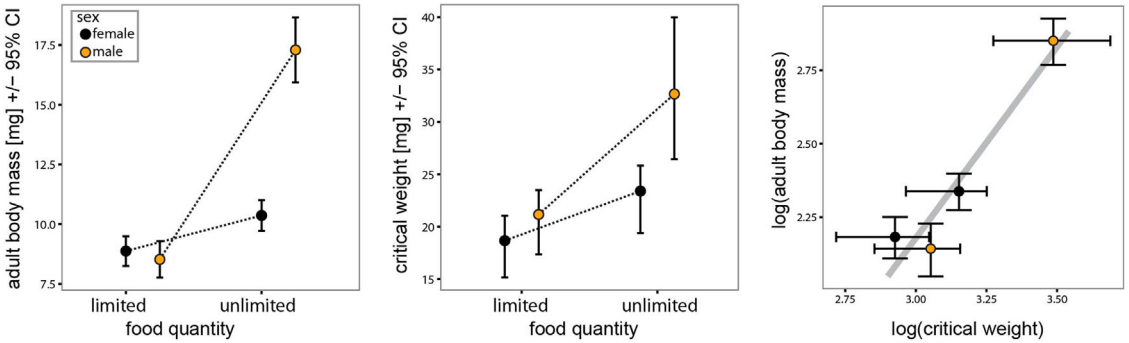

FIGURE 3 Adult body mass (left panel) and critical weight estimates (middle panel) show sex-specific plasticity in response to food availability. Critical weights and body size do not differ at limited food, but males increase their body size and critical weight more strongly than females with increasing food supply. Adult body mass and critical weights correlate strongly ($R^2 > 0.95$, $n = 4$), suggesting that critical weight is a major driver of intraspecific body size variation (log–log plot; right panel)

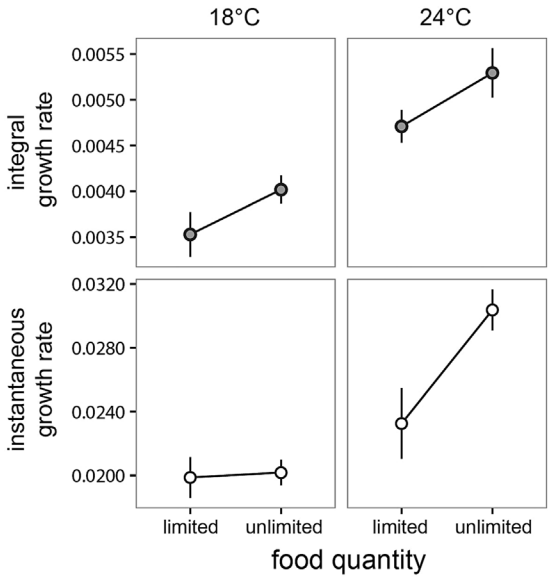

FIGURE 4 Integral growth rates are affected by temperature and food treatments, but there is no interactive effect. Similarly, instantaneous growth rates increase with temperature and food quantity, but in contrast to integral growth rates, instantaneous growth rates react more strongly to food limitation at high (24°C) than at low (18°C) temperature (also see Table S3). These contrasting patterns demonstrate that the two measures can differ substantially across environments such that caution is advised in choosing the appropriate measure when investigating insect growth

$F_{1,60} = 24.72, p < 0.001$). This resulted in a much greater peak mass of males than females once larvae stopped feeding (larval peak mass: $F_{1,61} = 11.09, p = 0.001$, Figure 1). SSD at the larval peak mass was more pronounced at unlimited food (larval peak mass; sex*food interaction: $F_{1,60} = 4.39$, $p = 0.040$, Figure 1). Surprisingly, independently of body mass, temperature, and food (marginally non-significant), males lost a disproportionate amount of their mass during the wandering stage (weight loss; sex effect: $F_{1,61} = 12.5$, $p = 0.001$), resulting in a less pronounced SSD in the pupal (pupal mass; sex effect: $F_{1,61} = 32.90$, $p < 0.001$, Figure 1) and adult stages (adult mass; sex effect: $F_{1,60} = 7.98$, $p = 0.010$, Figure 1). Even though at peak mass SSD was pronounced in all treatment combinations, adult SSD was only significant at unlimited food conditions (adult mass; sex*food limitation interaction: $F_{1,60} = 9.80$, $p < 0.001$, Figure 1). Males took overall longer to reach the adult stage (age at eclosion; sex effect: $F_{1,61} = 19.53$, $p < 0.001$) due to longer pupal development. Males were also more strongly affected by temperature than females (age at eclosion; sex*temperature interaction: $_{F1,61} = 4.01, p = 0.050$), because they spent more time in the larval stage (age at pupariation; sex effect: $F_{1,60} = 5.64$, $p = 0.021$).

Expectedly, instantaneous growth rates were always higher than calculated integral growth rates. Both estimates of biological growth correlate strongly ($r = 0.74$, $p < 0.001$), however. When analyzing differences between the two estimates of growth rates, we found that integral growth rates were generally more strongly affected by food quantity (type*food quantity: $F_{1,63} = 17.10$, $p < 0.001$). Differences between sexes and temperature treatments were more pronounced when using instantaneous growth rates (sex*type interaction: $F_{1,63} = 32.61$, $p < 0.001$; temperature*type interaction: $F_{1,63} = 74.34$, $p < 0.001$. Food quantity only had an effect on instantaneous growth at the high (24°C) but not at low (18°C) temperatures, whereas integral growth rates were always affected by food quantity, irrespective of temperature (type*food quantity*temperature interaction: $F_{1,63} = 28.03$, $p < 0.001$, Figure 4 and Table S3).

3.3 | Critical weight

While the sexes did not significantly differ in their critical weight at limited food (females: 18.66 mg [15.15, 21.05], males: 21.17 mg [17.35, 23.50]; [2.5th and 97.5th] percentiles are given; Figure 2), males had a higher critical weight at unlimited food (males: 32.67 mg [26.43, 39.98], females: 23.40 mg [19.39, 25.82]). That confidence limits overlap at limited but not at unlimited food in Figure 3 (center) suggests an interaction between sex and environmental conditions.

Apart from critical weight, we could not address the role of the ensuing terminal growth phase due to our measurement interval and sample size limitation. In fact, some estimates of critical weight exceeded the maximal larval peak weight of the averaged trajectory, likely an experimental artifact since critical weight and the growth trajectories were estimated in blocks given the amount work. Also, individual larvae might have been physically stressed by the daily measurements, potentially diminishing size increments. We are however confident in our estimates of growth trajectories, as they are very similar to those of earlier studies not tracking individuals (Blanckenhorn, 1999; Teuschl et al., 2007).

4 | DISCUSSION

S. stercoraria larvae respond to starvation depending on their larval weight, suggesting a *Drosophila*-like critical weight mechanism (cf., Stieper et al., 2008). Critical weight, representing the mass at which pupariation is initiated, was further associated with sexual size dimorphism and condition dependent body size plasticity in response to food availability (Figures 1–3). Therefore, (sex-specific) plasticity in critical weight likely plays a major role in generating body size variation of adult yellow dung flies. The detailed larval growth trajectories documented here reveal that even when adults do not differ in their body size, male and female larvae have dissimilar larval growth schedules. Thus, sexual size

dimorphism varies greatly in its extent throughout juvenile development, and the sexes are likely exposed to different selective pressures during the larval stages. In the following, we discuss the implications of size determination mechanisms and detailed larval growth assessments for the understanding of intraspecific body size variation and sexual size dimorphism.

The universal mechanism initiating metamorphosis, and thus terminating growth in all insects, is an increase in ecdysone titer during the final instar (Nijhout et al., 2014). In *Manduca*, *Bombyx*, and *Drosophila*, intraspecific adult size variation has been attributed in large parts to variation in critical weight, which is the weight (or an unknown correlate thereof; see below) at which a larva initiates ecdysone release (Nijhout et al., 2014). The commonness of the critical weight mechanism in insects is generally assumed, although empirical data are scarce. Our findings in *Scathophaga* advocate in favor of its prevalence. The mere phenotypic presence of a physiological switch-point does however not necessarily imply identical size-sensing mechanisms or homologous endocrine signals. Cuticle tension has been identified as a size-sensing mechanism in two hemipterans (Nijhout, 1984; Wigglesworth, 1940), but holometabolous insects appear to use different triggers. Studies on *D. melanogaster* found that the insulin-dependent growth of the prothoracic gland is associated with critical size sensing (Mirth, Truman, & Riddiford, 2005), while in *M. sexta* oxygen limitation due to the pre-assigned size of the tracheal system has been identified as a major driver in determining critical weight (Callier & Nijhout, 2011; but see Helm & Davidowitz, 2013). In contrast to the actual size-sensing triggers, the consequent endocrine cascades are much better resolved, although the effects of hormones can also vary between species (e.g., the role of JH and ecdysone in *Manduca* vs. *Drosophila* (Mirth et al., 2014)).

The timing of metamorphosis induction in *S. stercoraria* is environmentally plastic and its degree varies between the sexes, which produces severe fitness consequences in the adult stage. In *S. stercoraria* males are the larger sex, which is in stark contrast to *Manduca* and *Drosophila* which show female-biased SSD. The male-biased size dimorphism in the yellow dung fly is ultimately driven by stronger selection on male size relative to female size (Blanckenhorn, 2007, 2009). At the proximate level, we here demonstrated that males induce pupariation at a larger size than females (i.e., at a larger critical weight), in correspondence with the adult SSD. *Scathophaga* further shows an adaptive bail-out response to food limitation (Blanckenhorn, 1999), meaning that larval growth is accelerated yet shortened in order to reach the pupal stage prior to complete habitat depletion. Our data suggest that this response to food depletion is caused by a combination of heightened instantaneous growth rates and a lowered critical weight, which—all else being equal—reduce the larval period as well as peak weight. In addition, we found sex-specific plasticity in critical weights: males initiate pupariation at a larger size than females at unlimited food only, while the sexes did not differ in their critical weights under food limitation. This again corresponds well to the sex-specific plasticity in adult body size with males being the more plastic sex (Teuschl et al., 2007; Figures 1 and 3). Females thus seem to have a lower target size in good environments, in accordance with the existing SSD and the putative selection pressures leading to it (Blanckenhorn, 2007, 2009), implying that their size is more canalized relative to males. Critical weight and its plasticity thus account to a significant extent for the adaptive body size variation displayed by *S. stercoraria* (Blanckenhorn, 1998; Figure 3), and is therefore a likely target of sexual selection on male size and fecundity selection on female size. Recent findings in *D. melanogaster* suggest that variation in critical weight between sexes is linked to the insulin/insulin-like growth factor signaling pathway (Rideout, Narsaiya, & Grewal, 2015; Testa et al., 2013). Given that insulin is influenced by nutrition (Emlen, Szafran, Corley, & Dworkin, 2006; Shingleton, Das, Vinicius, & Stern, 2005), mediates growth and size, and also interacts with JH and ecdysone (Edgar, 2006), the insulin signaling pathway is a good candidate linking (sex-specific) plasticity to condition in *S. stercoraria*. Nevertheless, its impact on larval growth trajectories requires explicit investigation, particularly in light that *Drosophila* and *Scathophaga* diverged about 50 mya (Wiegmann et al., 2011).

In insects, the evolution of sexual size dimorphism and body size plasticity are primarily studied in adults even though structural body size variation must be induced before the adult cuticle is fully sclerotized. Potential costs of adult size variation thus already arise during the larval or nymphal stages, which is why the mechanisms are crucial to understand the evolution of sexual size dimorphisms and body size in general (Stillwell et al., 2014; Tammaru & Esperk, 2007; Testa et al., 2013; Vendl, Kratochvíl, & Šípek, 2016). SSD has been attributed to variation in the number of instars between the sexes (Esperk, Tammaru, Nylin, & Teder, 2007), sex-specific growth rates (Blanckenhorn et al., 2007; Rohner, Blanckenhorn, & Puniamoorthy, 2016; Vendl et al., 2016), variation in development time (Teder, 2014; Rohner et al., 2016), or unequal (post-eclosion) weight loss (Molleman et al., 2011; Testa et al., 2013; see below). We found that in *S. stercoraria* sexual dimorphism in larval peak mass arises due to unequal rates and durations of the initial instantaneous growth, with males growing faster and for a prolonged period. Males were always larger than females prior to the wandering stage. Interestingly however, males also lost significantly more weight after growth ceased (on average: 24.6% in males vs. 21.6% in females: Figure 1). This loss was independent of their body size and especially striking at limited food conditions, with larvae showing strongly male-biased SSD whereas the emerged adults were monomorphic in size,

reaffirming that larval growth trajectories differ between the sexes. Since adult size is under strong selection in males, this weight loss is doubtlessly costly and begs for scrutiny. Most of this weight loss is certainly due to the purging of gut content, but further metabolic costs and the continued development during the wandering stage certainly add to it (Reim, Kaufmann, & Blanckenhorn, 2009). Testa et al. (2013) found that female *Drosophila* larvae, which are larger than males, also lost more weight during the wandering stage. The authors hypothesized that the instantaneous growth rate differences of male and female larvae might be linked to the growth of the imaginal discs, which continue to develop during the wandering stage. If this holds true, imaginal discs of the faster growing sex would generally deplete a larger amount of stored resources. Indeed, on average *S. stercoraria* males grow faster than females. However, weight loss neither correlated with the instantaneous growth rate nor with peak mass in our study, suggesting a different mechanism. Alternatively, sexually dimorphic timing of growth and development of imaginal discs could be responsible for the unequal weight loss of the sexes documented here. Although in *Drosophila* male gonads grow slower than their female counterparts during the late larval stage (Kerkis, 1931), it remains possible that male *S. stercoraria* invest more in growth and development of imaginal tissue during the late larval stages, which could explain why males spend less energy during the pupal stage (Reim et al., 2009).

As demonstrated here and elsewhere (Blanckenhorn, 1999; Tammaru & Esperk, 2007; Tammaru et al., 2010; Teuschl et al., 2007), detailed individual growth trajectories harbor great potential in untangling variation in larval growth. Such assays demand tremendous efforts and may not be applicable in some taxa or certain environments. As a result, integral growth rates estimated from final body sizes and the corresponding egg-to-adult development times are widely in use (Figure 1). Instantaneous growth rates were on average 6.7 ± 0.2 SE times higher than integral rates in females and 7.9 ± 0.3 SE times higher in males. We further found that the two different estimates of growth rate differ in their response to food limitation. Instantaneous growth rates reacted much more strongly to food shortage at high than at low temperatures while the effect of food quantity on integral growth rates was independent of temperature (three-way interaction; Figure 4). This is not an issue of statistical power; rather, the two estimates greatly differ in their conceptual and biological meaning. While instantaneous growth rates estimate the speed of growth during the (more or less) continuous quasi-exponential initial growth phase, integral growth rates integrate not only the total amount of gained weight but also the amount of mass lost throughout juvenile development, including the process of inter-instar molting. Nevertheless, the two measures correlate quite strongly ($r = 0.7$), which implies that the initial rate of growth scales roughly with the subsequently realized growth. However, as demonstrated here, the shape of growth trajectories can differ consistently between sexes and environments, consequently introducing potentially systematic biases when analyzing integral estimates. Obviously this can, but must not necessarily be problematic. Special caution is surely appropriate if integral growth rates are compared among species, especially if taxa differ in their ecology. Our main point here is that integral growth rates clearly depict a different biological property than instantaneous growth rates, and this inequality may not always be appreciated sufficiently (Esperk et al., 2013; Tammaru & Esperk, 2007).

In summary, we here shed light on the physiological and developmental underpinnings of condition dependent sex-specific body size plasticity and showed that critical weight, a major size determinant, is likely a major driver responsible for sex-specific plasticity and ultimately sexual dimorphism in the yellow dung fly and, probably, insects in general. Our study further suggests that critical weight(-like) mechanisms are indeed common in insects. We also demonstrated the usefulness of detailed larval growth trajectories for the understanding of the ontogeny of sex-specific plasticity in growth, as opposed to simpler estimates of integral growth. Future research should aim at uncovering the proximate causes and the evolvability of sex-specific plasticity in other non-model species in order to obtain a more general understanding of how adult body size variation arises.

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SUPPORTING INFORMATION

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Does thermal plasticity predict clinal variation in wing size and shape? – An inter- and intraspecific comparison in two sepsid flies

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Key words: geometric morphometrics, cogradient, countergradient, Diptera, Sepsidae, thermal plasticity, latitudinal clines, wing loading, dispersal

Abstract

Given its profound effect on biological systems, temperature often elicits phenotypic plasticity as well as quantitative genetic differentiation. If genetic and plastic responses to temperature are adaptive, they should be related in magnitude and form, a pattern that should evolve repeatedly in different lineages. We here quantified the hypothesized positive relationship between quantitative genetic latitudinal variation in wing loading and wing shape and their thermal plasticity in two closely related sepsid flies with contrasting sexual size dimorphism (Diptera: Sepsidae). Common garden rearing revealed that wing loading decreases with latitude in both species, likely driven by selection for increased dispersal capacity in the cold. Thermal plasticity for wing loading was however hump-shaped, suggesting a more complex relationship between plasticity and genetic differentiation. Although wing allometry, sexual dimorphism and thermal plasticity show similar patterns in both species, latitudinal differentiation only mirrors thermal plasticity in *Sepsis punctum*, but not in *Sepsis fulgens*. Such discrepancies may be driven by variation in gene flow and demography; hence, our results support the notion that genetic wing shape differentiation may be complex and idiosyncratic, even among ecologically similar closely related species.

Introduction

Temperature causes predictable plastic responses and consequently repeatedly elicits the evolution of genetic differentiation in life history, morphology and behaviour throughout the tree of life (Alho, et al. 2010; Allen 1877; Atkinson 1994; Atkinson, et al. 2006; Bergmann 1847; Clauss, et al. 2013; Ray 1960; Schilthuizen and Kellermann 2014; Taylor, et al. 2016; Zaidi, et al. 2017). This is primarily due to the temperature dependency of biochemical processes inherent to all biological systems, which has direct consequences for an individuals' fitness (de Jong and van der Have 2009; Hochachka and Somero 2014). Given its profound effect, and ongoing global climate change, the great scientific interest in understanding how organisms adapt to temperature, in the short as well as long the term, is not surprising. In this context, patterns of thermal plasticity and genetic differentiation along latitude, as well as their relationship, have received particular attention (Phillimore, et al. 2010; Schilthuizen and Kellermann 2014; Stoks, et al. 2014).

In insects and other cold-blooded (ectothermic) taxa, plastic responses to temperature are pervasive. In most species, higher temperatures lead to reduced body size (temperature-size-rule), with associated plastic responses in growth (faster) and development time (shorter; Atkinson 1994; de Jong and van der Have 2009). However, temperature can also drive the evolution of adaptive genetic differentiation. Examples include the evolution of latitudinal or altitudinal clines in development times and voltinism (Zeuss, et al. 2017; Blanckenhorn, et al. 2018), melanisation (Karl, et al. 2009), or hibernation behaviour (Demont and Blanckenhorn 2008). Perhaps unsurprisingly, therefore, comparisons frequently reveal evidence for a relationship between plasticity and genetic differentiation. In general, genetic and plastic responses do not need to be related, for instance if gene flow hampers local adaptation, if the costs of being plastic are minor, or if patterns are not driven by selection altogether (Flatt 2016). However, if selection along latitude is similar to the selective pressures that shape ther-

mal plasticity (i.e. selection is synergistic), quantitative genetic differentiation from south to north is expected to mirror the plastic response from hot to cold temperatures (Phillimore, et al. 2012). Such patterns of covariance thus establish *co*gradients (Conover, et al. 2009; Conover and Schultz 1995; Falconer 1990). If on the other hand, selection along latitude and thermal plasticity oppose each other, genetic differentiation is expected to counteract the plastic response, leading to *counter*gradient variation (also referred to as "genetic compensation": Grether 2005). Such patterns may for instance arise if plasticity causes unfavoured phenotypes across an environmental gradient. Stabilizing selection may then canalize a phenotype via compensatory genetic differentiation in the opposite direction (as is frequently discovered for insect growth rates; e.g.: Blanckenhorn and Demont 2004; Kivela, et al. 2012; Meister, et al. 2017). Both co- and countergradient variation are frequently found in insects (reviewed in Conover, et al. 2009), and are helpful in understanding how phenotypic variation arises and in predicting adaptation to temperature in the future (Phillimore, et al. 2010).

It has been argued that linear traits, such as body size or development time, may not be well suited to compare plasticity to genetic differentiation, because they only vary along one dimension (see Pitchers, et al. 2013). Accordingly, chances are high that plastic and genetic responses appear qualitatively similar even in the absence of a common underlying selective driver. In contrast, studying traits with high dimensionality can alleviate such limitations provided that the traits under scrutiny are not strongly genetically correlated. When comparing the effects of genetic differentiation and plasticity in *n*-dimensional space, spurious qualitative accordance becomes less likely, such that their degree of covariation can be quantified.

In this regard, plastic and genetic shape variation of insect wings has been scrutinized in particular. Being the prime agent of dispersal in many pterygote species, wings are likely to be targets of natural selection (Gilchrist, et al. 2000). Although wings are doubtlessly important for foraging and evasion from predators (or fly flaps for that matter), wing shape and size may be especially important in the context of thermoregulation. In contrast to warm-blooded (endothermic) vertebrates, small-bodied insects have only limited intrinsic capacity to regulate their body temperature (Harrison, et al. 2012). Hence, the majority of insects regulate their temperature by behavioural means (Chown, et al. 2004; Stevenson 1985). Small winged insects, such as fruit flies, therefore use their flight ability to make best use of their heterogeneous habitats (Dillon, et al. 2009). However, the capacity for flight is hampered in the cold (Dillon and Frazier 2006; Frazier, et al. 2008). This biophysical constraint can be overcome to some extent by increasing relative wing size, thereby generating more lift and allowing for take-off at lower temperatures. Such temperature-dependent plasticity, as well as genetic latitudinal and altitudinal variation, in wing loading (i.e. the ratio between body weight and wing area) has been found repeatedly within as well as across species (Azevedo, et al. 1998; Gilchrist and Huey 2004; Rohner, et al. 2015; Rohner, et al. 2018; Stalker 1980). Similar arguments have also been forwarded concerning the shape of the wing, which again shows plastic as well as clinal variation (Gilchrist and Huey 2004; Gilchrist, et al. 2001; Pitchers, et al. 2013).

Not surprisingly, most of the literature on latitudinal clines and thermal plasticity in wing shape focusses on a few species of *Drosophila* (e.g.: Debat, et al. 2003; Hoffmann and Shirriffs 2002; Imasheva, et al. 1995; Pitchers, et al. 2013; but see a recent study on the yellow dung fly by Schäfer, et al. 2018). This is probably primarily due to the ease of laboratory rearing permitting direct assessment of genetic divergence. However, although the numerous studies on drosophilids have improved our understanding on how wing shape and size vary and evolve, it remains unclear at this point to which extent such patterns are also found in other species.

Revisiting two independent common garden rearing experiments by Berger et al (2013) and Roy et al (2018), we here directly investigate the relationship of thermal plasticity and (genetic) latitudinal differentiation in relative wing size and shape in two species of black scavenger flies with contrasting sexual size dimorphism (Diptera: Sepsidae). We first quantify plastic and genetic variation in wing shape and size, to test whether latitudinal variation mirrors thermal plasticity, and then investigate the extent to which patterns correspond across the two species. If patterns are mostly driven by temperature, we expect clinal (i.e. genetic) variation in wing shape and size to mimic the plastic thermal responses. We also expect that different species show similar, convergently evolved clinal variation.

Materials & Methods

Sepsis fulgens Meigen, 1826 and *Sepsis punctum* (Fabricius, 1794) are both common, erytopic species of black scavenger flies (Diptera: Sepsidae) found throughout Europe (Ozerov 2005; Pont and Meier 2002). Being small, multivoltine acalyptrates developing in decaying organic substrates, sepsids are expected to be under similar ecological selection pressures as drosophilids, although previous studies demonstrated contrasting latitudinal clines for life-history traits (e.g. body size: Puniamoorthy, et al. 2012; Roy, et al. 2018). Although *S. punctum* has been argued to be adapted to warmer climates, these two rather closely related species can frequently be found in the same microhabitats, where both species preferentially use vertebrate dung as breeding substrate (mostly cattle dung or dung heaps, but also pig or dog excrements) (Pont and Meier 2002; Rohner and Bächli 2016; Rohner, et al. 2015).

Laboratory isofemale lines of both species were established using offspring of wild-caught, gravid females from 9 populations for *S. fulgens* (Roy, et al. 2018; fig. 1a), or by using the first filial generation of females emerging from individual dung traps deposited in the field at 7 European locations (*S. punctum*; fig. 1a). Upon establishment, isofemale lines where provided with a continuous supply of water, sugar and fresh cow dung and subsequently reared for multiple generations under laboratory conditions.

Common garden rearing

To test for genetic latitudinal variation and thermal plasticity in (relative) wing size and shape, isofemale lines of all populations were reared under common garden conditions using several temperature treatments. Note that we here revisit a subset of animals that were reared in two temporally separated common garden settings in previous studies (*S. punctum*: Berger, et al. 2013; *S. fulgens*: Roy, et al. 2018). These individuals were frozen with a drop of water to avoid evaporation and stored until dissected for morphometric measurements. In both common garden experiments, isofemale lines were provided with a small amount of homogenized, previously frozen cow dung for oviposition. After 24h, the dung was removed to retrieve eggs. Larvae were then provided with standardized cow dung *ad libitum*, preventing larval competition for food and space and hence limiting environmental effects on phenotypic variation.

In *Sepsis fulgens*, each line was reared at four temperatures (12, 18, 24, 30°C), while lines of *S. punctum* were similarly reared at five temperatures (15, 18, 23, 28, 31°C). Note that although the temperature spectrum and the number of treatments do not correspond fully between species, patterns of quantitative genetic differentiation can still be compared between species when only considering individuals reared at 18°C, as this temperature regime was applied in both species. Other effects of the two experimental blocks cannot be controlled for; nevertheless, this should not affect our ability to compare morphological patterns of plasticity and differentiation within species.

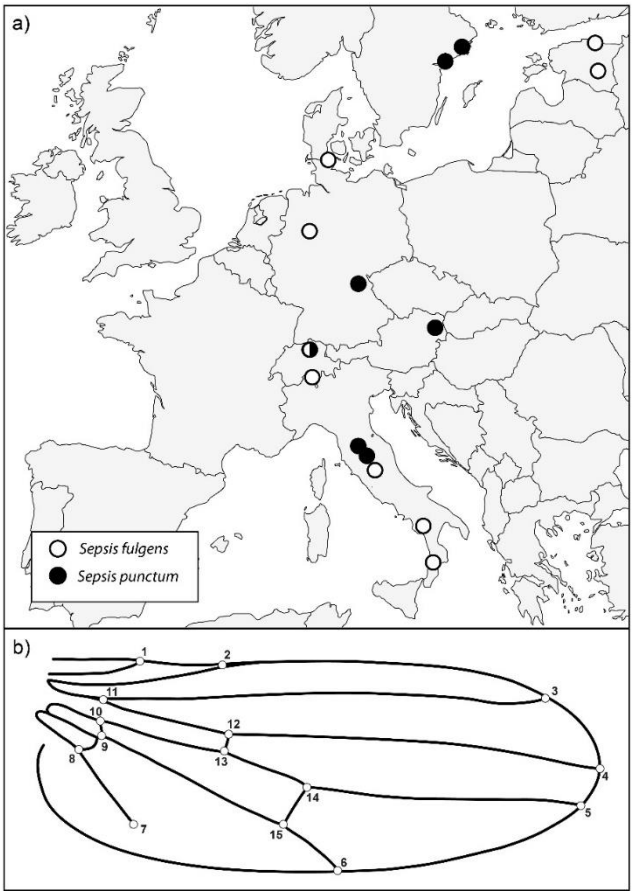

Figure 1: a) Population sampling for S. fulgens (open circles) and S. punctum (filled circles) across Europe. b) shows the landmarks used to quantify genetic as well as plastic shape variation.

Table 1: ANOVA tables (type III sums of squares) for thermal plasticity in wing centroid size, thorax length and wing loading show consistent non-linear reaction norms in both species (temperature^2-term). The sex × temperature^2 - interaction was not significant throughout and hence removed.

	wing centroid size				thorax length				wing loading			
Sepsis punctum	**MS**	**ddf**	**F**	**P**	**MS**	**ddf**	**F**	**P**	**MS**	**ddf**	**F**	**P**
temperature	0.072	619.0	5.15	0.024	0.236	592.7	50.94	<0.001	0.162	591.0	186.52	<0.001
temperature^2	0.856	619.0	61.59	<0.001	0.480	593.3	103.62	<0.001	0.045	591.3	51.92	<0.001
sex	1.162	619.0	83.66	<0.001	1.205	591.4	260.43	<0.001	0.051	591.4	59.00	<0.001
sex × temperature	0.884	619.0	63.60	<0.001	0.448	591.4	96.90	<0.001	0.037	591.0	42.04	<0.001
Sepsis fulgens	**MS**	**ddf**	**F**	**P**	**MS**	**ddf**	**F**	**P**	**MS**	**ddf**	**F**	**P**
temperature	2.269	277.2	52.44	<0.001	1.050	278.3	58.17	<0.001	0.080	278.5	25.03	<0.001
temperature^2	2.611	277.2	60.35	<0.001	1.060	278.3	58.74	<0.001	0.144	278.4	45.29	<0.001
sex	0.764	277.2	17.66	<0.001	0.896	278.5	49.63	<0.001	0.128	278.4	40.29	<0.001
sex × temperature	0.137	277.2	3.16	0.077				n.s.				n.s.

Morphometric measurements

For morphometric analysis, we removed the right wing of two individuals per sex, isofemale line, temperature, population and species, and mounted it on a glass slide using Euparal. The dissected wing, as well as the thorax (lateral view) were photographed using a Leica DFC490 camera mounted on a Leica MZ12 microscope. The length of the thorax was measured as the cumulative length of the scutum and the scutellum using digitized landmarks derived from tpsDig vers. 2.14 (Rohlf 2009).

To quantify wing shape, we digitized 15 landmarks, again using tpsDig (see fig. 1b for a visual representation of the landmarks), which were used to calculate centroid size (a composite measure of overall wing size: Klingenberg 2016), and retrieved Procrustes-transformed coordinates using the function *gpagen()* of the R-package *geomorph* (Adams and Otárola-Castillo 2013). As an estimate of wing loading, we divided thorax length^3 (which correlates well with total weight, unpublished data PTR) by the centroid size^2 (i.e. wing area).

Statistical analysis

To assess latitudinal clines within species, we regressed the average of thorax length and wing centroid size per isofemale line against latitude using the (random) population term as error term (with the function *lmer* in R: Kuznetsova, et al. 2017). To test for clinal variation in wing loading, we fitted wing size as a function of latitude (again using population as the error term) with thorax length as covariate. We restricted these analyses to individuals raised at 18°C, allowing direct comparison between the species. Note that *S. punctum* and *S. fulgens* are closely related, but not sister species (Zhao, et al. 2013). They differ in overall body size (*punctum* is considerably larger) and also in sexual size dimorphism (larger females in *fulgens*, larger males in *punctum*). Any clinal patterns are hence likely driven by convergent evolution and not due to shared ancestry.

To test for thermal plasticity, we also used linear mixed models with population as random effect. As thermal reaction norms are usually non-linear, we fitted sex-specific line means as a function of temperature, temperature^2, and sex, as well as the sex × temperature- and sex × temperature^2-interactions. Clinal variation in plasticity was tested by adding latitude and its interaction with temperature as well as temperature^2. Non-significant interaction terms were removed from the final model.

We tested for latitudinal variation in wing shape within species by using the multivariate regression approach as implemented in MorphoJ (Klingenberg 2011). We first averaged the Procrustes-transformed wing coordinates by population and regressed these averages against latitude. The statistical significance of these multivariate regressions was assessed using randomization tests (using 10,000 random samples). We used the same approach to test for allometric shape variation, thermal plasticity and sexual shape dimorphism, but used isofemale line means as level of comparison.

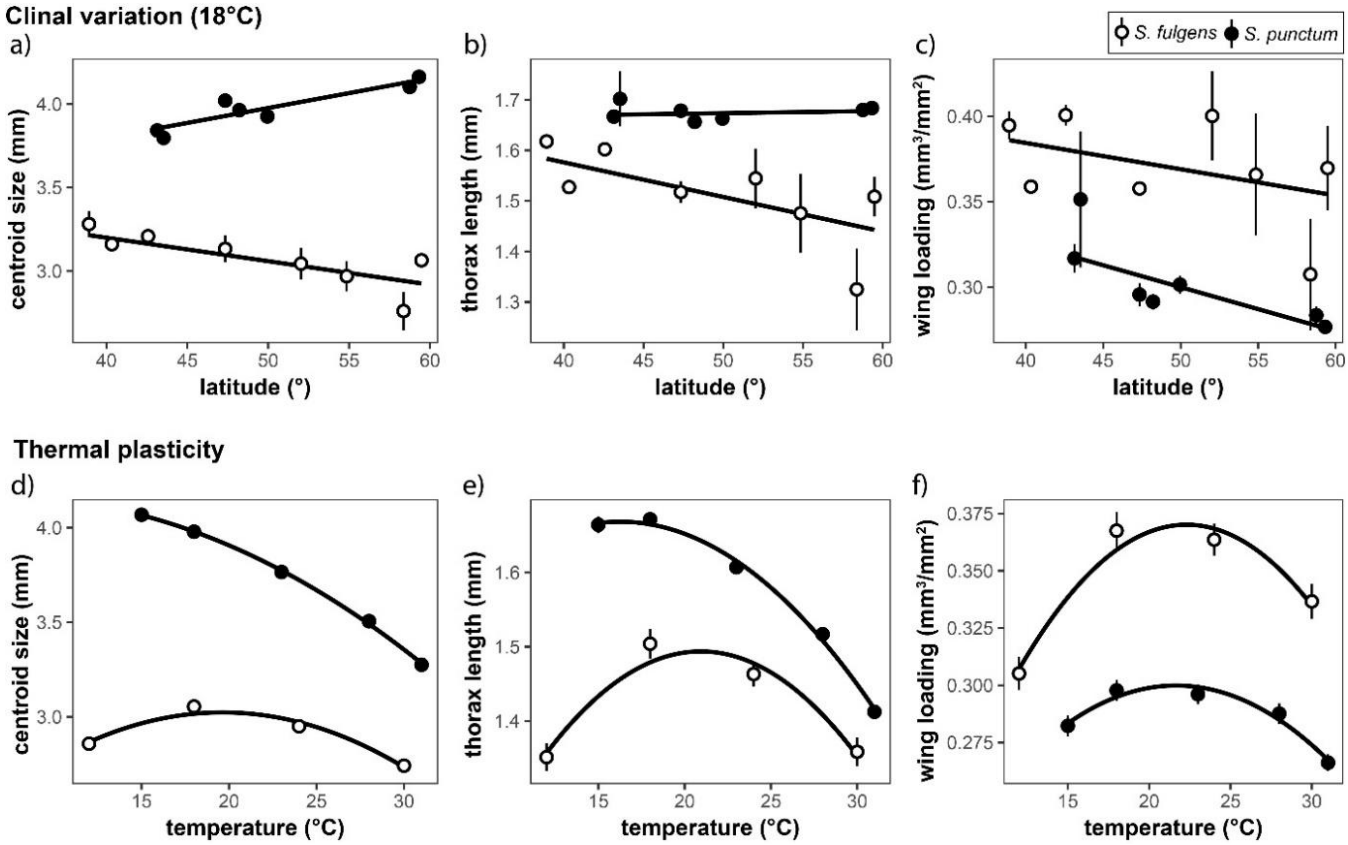

Figure 2: Clinal variation (a-c) and thermal plasticity (d-f) for wing centroid size, thorax length and wing loading for S. fulgens (open circles) and S. punctum (filled cirles). While wing centroid size (a) and thorax length (b) differ qualitatively between S. punctum and S. fulgens, both species show a decrease in wing loading towards higher latitudes (c). Temperature effects on wing size (d), thorax length (e) and wing loading (f) were non-linear and pronounced.

To compare the latitudinal genetic differentiation with the direction of the plastic response to temperature, we calculated correlations among their shape deformation vectors. We included sex and centroid size as covariates to control for allometry and sexual shape dimorphism. To this end, we used a Bayesian multivariate generalized linear mixed effects model with Markov Chain Monte Carlo sampling (R-package *MCMCglmm* (Hadfield 2010)) to first estimate the effects of each variable on shape simultaneously (as in Schäfer, et al. 2018). Instead of using Procrustes-transformed coordinates directly, we used their Principal Components (PCs). This was done because PCs are by definition orthogonal and hence cause no computational issues related to multicollinearity (in sharp contrast to Procrustes data). Because Procrustes superimposition results in a deficiency of four ranks, we only fitted the first 26 PCs (15 landmarks × 2 coordinates - 4 deficient ranks). MCMCglmms were fitted separately for both species using isofemale lines and populations as random effects. The off-diagonal elements of the covariance matrix were set to zero (using the *idh()* function of MCMCglmm) given the orthogonal structure of the PCs at the individual level. Uninformative priors based on population identity were used for the residual and both random covariance matrices (R, G1, G2: v = 0.10-6). Models were run for 220,000 iterations using a thinning interval of 100, with the first 20,000 iterations discarded (burn-in), resulting in 1000 uncorrelated posterior estimates stored for further analysis.

Although latitude correlates with major climatic variables other than temperature, in particular season length, temperature is the most important climatic variable for small, fast developing multivoltine insects such as drosophilids, sepsids and other small Dipterans (Blanckenhorn and Demont 2004; Shelomi 2012). Consequently, we in the following interpret any genetic variation associated with latitude mostly as a correlate of temperature or its variability.

Results

Clinal variation and thermal plasticity in wing size and wing loading

Wing (centroid) size increases at higher latitude in *S. punctum* ($F_{1,5.29}$ = 25.48, P = 0.003, fig. 2a), but decreases with latitude in *S. fulgens* ($F_{1,7.35}$ = 7.92, P = 0.025, fig. 2a). Thorax length showed no latitudinal variation in *S. punctum* ($F_{1,5.1}$ = 0.19, P = 0.668, fig. 2b), but a marginally non-significant decrease with latitude in *S. fulgens* ($F_{1,7.70}$ = 4.37, P = 0.071, fig. 2b). However, even though the patterns in wing size and thorax size differ qualitatively between species, wing loading showed a consistent decrease towards the poles in both species, though this relationship was marginally non-significant in *S. fulgens* (*S. punctum*: $F_{1,5.07}$ = 23.44, P = 0.005, *S. fulgens*: $F_{1,7.93}$ = 4.56, P = 0.066, fig. 2c).

Wing size, thorax length and wing loading showed a non-linear relationship with developmental temperature (temperature^2: all P < 0.001, table 1), a typical shape of thermal reaction norms (Chapman, et al. 2013). Thermal plasticity of centroid size showed clinal variation in *S. punctum*, for which the non-linearity of the thermal reaction norm steadily increased with latitude (latitude × temperature^2 - interaction: $F_{1,24}$ = 21.9, P >0.001; fig. 3). For all other traits, the latitude × temperature- and the latitude × temperature^2- interactions were non-significant.

S. punctum and *fulgens* are sexually dimorphic for all traits measured. While males had larger wings, longer thoraces and lower wing loading in *punctum*, we find the opposite patterns in *S. fulgens* (table 1) in which females are the larger sex (cf. Rohner, et al. 2016).

Clinal variation and thermal plasticity in wing shape

We found significant allometric variation, thermal plasticity and sexual dimorphism for wing shape in both species (all P ≤ 0.014). In contrast to *S. punctum,* which exhibits latitudinal variation in wing shape (P = 0.043, n = 7), *S. fulgens* does not show significant levels of genetic differentiation along latitude (P > 0.5, n = 9).

When comparing vectors of model coefficients between the species, i.e. the alignment of shape change observed in the two species in response to a particular explanatory variable, we found the effects of temperature on shape (r = 0.33 [0.19, 0.46], fig.4b) to correlate moderately but significantly between species, while shape allometry (r = 0.87 [0.76, 0.92], fig.4b) and sexual shape dimorphism (r = 0.60 [0.42, 0.92], fig.4b) are highly similar across species. The genetic differentiation along latitude showed similar patterns in *S. punctum* and *S. fulgens*, but this correlation was not significantly different from nil (r = 0.58 [-0.07, 0.83], fig.4b), which is unsurprising given the lack of significant clinal variation in *S. fulgens* and the low statistical power associated with estimating these correlations (based on population rather than isofemale line means).

Thermal plasticity as well as sexual shape dimorphism were to some extent dependent on allometry in both species, while latitudinal differentiation was not (see fig. 4a). The latter result was unexpected, given that wing size shows clinal variation in both species. Interestingly, while the effect of latitude on shape is not correlated with the effect of temperature in *S. fulgens* (r = 0.08 [-0.47, 0.51], fig. 4a), it is negatively correlated with plasticity in *S. punctum* (r = -0.59 [-0.74, -0.37], fig. 4a). As latitude is inversely related to temperature (colder at higher latitudes), the genetic latitudinal cline follows the pattern of thermal plasticity in wing shape within populations, i.e. northern populations show wing shapes similar to flies reared at cool temperatures.

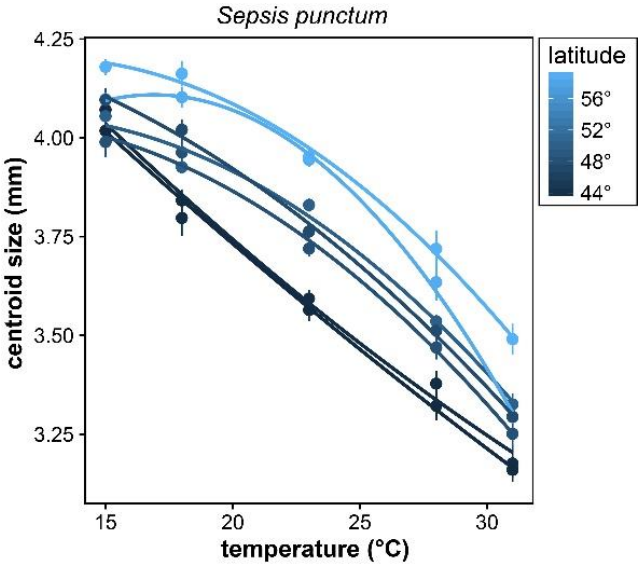

Figure 3: Clinal variation in phenotypic plasticity for wing centroid size in S. punctum.

Discussion

The relationship between genetic differentiation and plastic responses has been subject to considerable scrutiny. We here investigated the consistency of the relationship between thermal plasticity and latitudinal differentiation in wing size and shape across two closely related dipterans. Wing loading

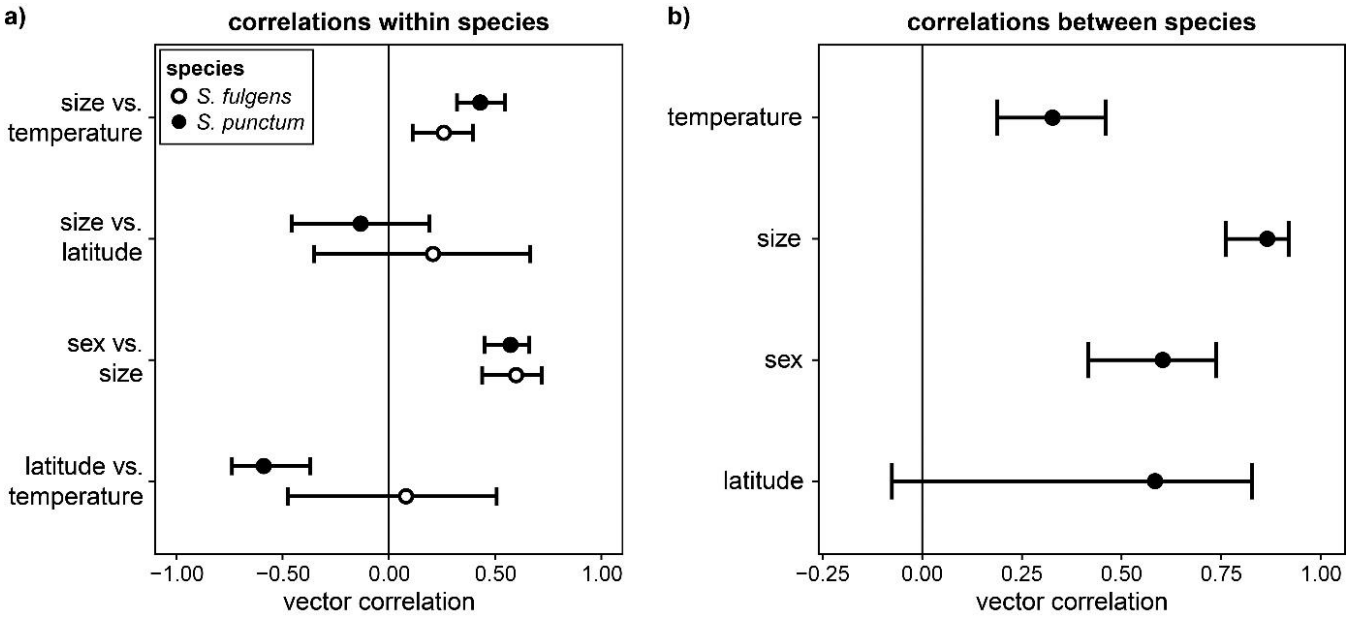

Figure 4: Correlations between the vectors of model coefficients (± corresponding 95% credibility intervals). A separate multivariate MCMCglmm was fitted for each species. (a) depicts the similarity of effect between different variables on wing shape, while (b) shows the similarity of effect between species.

showed the typical hump-shaped (nonlinear) thermal plasticity, as well as clinal variation in both species. As our common garden study minimized extraneous environmental variation, these clines are genetic, and thus provide evidence of convergent evolution. This suggests an adaptive response to selection for increased dispersal capacity in colder and/or more variable environments (c.f. Stalker 1980; see below). The relationship between genetic differentiation and plasticity of wing loading however is more complex, as thermal reaction norms were highly non-linear whereas the latitudinal clines were not. Furthermore, the association between evolved latitudinal wing shape clines and their plasticity was inconsistent between species. While *S. punctum* shows pronounced clinal patterns aligning with the effect of temperature (i.e. cogradient variation), *S. fulgens* only showed minor (if any) latitudinal shape variation that did not correspond to the plastic response. As a whole, our results suggest that wing size evolves independently of wing shape and that genetic differentiation not necessarily follows the plastic response.

Plasticity and genetic differentiation in wing loading

Even though wing as well as thorax size clines differed qualitatively between species (fig. 2a,b), both *S. punctum* and *S. fulgens* surprisingly showed decreasing wing loading towards higher latitude (fig. 2c). As lower wing loading has been argued to provide better dispersal capacity in the cold (Azevedo, et al. 1998; Frazier, et al. 2008; Stalker 1980), and the decline in wing loading with latitude was seen in both species, the pattern obtained seems to be of adaptive value (c.f. Endler 1977). Moreover, a corresponding altitudinal gradient in wing loading has been described in sepsids (Rohner, et al. 2015), and similar and consistent intra- as well as interspecific clines have been demonstrated for wing loading in *Drosophila* (Azevedo, et al. 1998; Rohner, et al. 2018). These recurring patterns argue in favour of a widespread adaptive pattern, at least among small dipterans. If so, this implies that wings are not well-suited as a surrogate trait for studying body size variation along latitudinal clines (as is frequently done when assessing Bergmann's or James' rules: Shelomi 2012) because they likely follow their own patterns as main dispersal agents.

Following earlier studies (e.g. Azevedo, et al. 1998), we expected wing loading in sepsids to increase with temperature. When only considering cold to moderate temperature treatments, this prediction was upheld, suggesting cogradient variation across Europe. When considering the full reaction norm, however, we found very low wing loadings at higher temperatures, a non-linear pattern present in both species. One could thus argue that selection on dispersal may not only drive decreased wing loading in cold habitats, but also in environments that are prone to over-heating. In both cases, heightened dispersal capacity should increase the efficiency of thermoregulatory behaviour and dispersal. However, such hump-shaped nonlinearities are

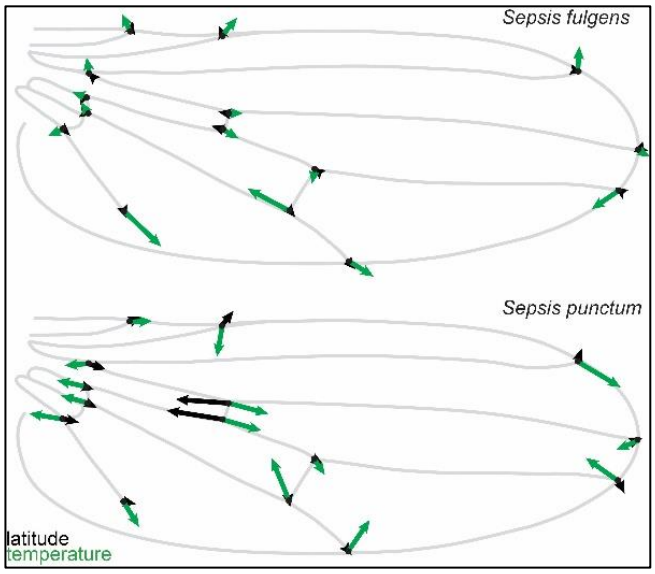

Figure 5: Shape deformations associated with temperature (black) and latitude (green) in both species. While thermal plasticity does not well correspond to latitudinal shape differentiation in S. fulgens, clinal variation mirrors the plastic response in S. punctum. Note that because average temperature regimes decrease with latitude, the apparent opposing patterns actually demonstrate a positive association between the plastic and the genetic response.

the rule, rather than the exception, for thermal performance curves (Chapman, et al. 2013; Chown, et al. 2004). Organisms usually perform best at some "optimal", intermediate temperature (Angilletta 2009), hence phenotypes measured at the edges of their intrinsic temperature tolerance are always difficult to interpret, as they may reflect physiological constraints related to thermal stress, leading to trait decanalization during development. Note, however, that the upper thermal limit (in terms of development and survival) of the two spesid species studied here lies beyond 30°C. Further, the allometry between thorax length and body weight itself might be temperature-dependent. Our approach of using thorax length^3 as an estimate of overall body weight might therefore introduce artefacts. Consequently, for now, the adaptive value of thermal plasticity, and its relationship to clinal variation, in wing loading must remain unclear but warrants further scrutiny.

Plasticity and genetic differentiation in wing shape

Plastic as well as genetic responses in wing shape have been described in *Drosophila sp*. While in these studies clinal variation in wing shape is often attributed to systematic differences in local selective regimes (e.g. Hoffmann and Shirriffs 2002; Moraes and Sene 2007; Pitchers, et al. 2013), genetic differentiation along latitudinal clines are not ubiquitous and can arise due to drift and demography as well (Flatt 2016; Schäfer, et al. 2018). Here, we found evolved latitudinal differentiation in *S. punctum* that correlated with the plastic response. Northern *punctum* populations consequently show wing shapes similar to flies reared at cold temperatures. Such a cogradient is expected if the plastic and genetic responses are adaptive and driven by synergistic selection pressures (or linked to a locus under selection). However, the observed pattern fits at least phenomenologically also to the predictions of genetic accommodation, where ancestral plasticity becomes genetically canalized within populations (Crispo 2008; Moczek 2007; Waddington 1952; West-Eberhard 2003). Differentiating between accommodation and cogradients is difficult. Due to the canalizing nature of genetic accommodation, the total phenotypic variation should decrease, a pattern opposite to the effect of cogradient variation, which should amplify the observed variance (Conover, et al. 2009). Given lack of information on the ancestral thermal plasticity, we cannot assess this critical difference and contend that evidence for either process remains equivocal.

In contrast to *S. punctum*, we did not find latitudinal differentiation in *S. fulgens*. Note however that, albeit not significant, the shape changes associated with latitude correlated quite strongly between species. The lack of significance in *S. fulgens* may hence be due to very small effect sizes and a limited statistical power when regressing population means (n = 9) against latitude. Nevertheless, the latitudinal effect in *S. fulgens* did not relate to thermal plasticity. This absence of a cogradient suggests that the evolutionary processes driving wing shape differentiation are complex and not necessarily similar across even closely related species. As the two focal species are rather similar in their distribution and ecology, it seems doubtful that selection on wing shape strongly contrasts between them. However, the potential for local adaptation greatly depends on the level of gene flow between populations (Crispo 2008). Previous data demonstrate strong genetic differentiation in European *S. punctum* between populations north and south of the Alps, as well as significant isolation by distance across northern parts of the species' distribution (Puniamoorthy 2013). Because *S. punctum* is rather rare at high altitudes (Rohner, et al. 2015), such genetic signatures are likely driven by limited gene flow across the alpine region, and may well relate to the species' colonization histories after the last glaciation. In contrast, *S. fulgens* is common also at high

altitudes (Rohner, et al. 2015). Although we currently lack information on the underlying population structure in this species, we suspect that the Alps should not pose a major barrier to gene flow, which would lead to only low levels of genetic differentiation across Europe (as in the closely related *S. cynipsea*: Kraushaar, et al. 2002). This would potentially hamper the potential for local adaptation, preventing the establishment of clines unless counteracting selection is strong. The interspecific variation in shape clines might hence be explained by the underlying population structure and demography (cf. Schäfer, et al. 2018). If so, that would also imply that selection on wing shape is rather weak compared to selection on wing loading (for which we see latitudinal clines).

The effect of size on phenotypic differentiation

Due to pronounced sexual size dimorphism and thermal plasticity of size, sexual shape dimorphism and temperature plasticity are to some extent driven by allometry. Nevertheless, both sex and temperature lead to shape variation independent of allometry, as found in other species (Gidaszewski, et al. 2009). Note that sexual size dimorphism is male-biased in *S. punctum* and female-biased in *S. fulgen*s. Even though sexual dimorphism in shape contrasts qualitatively, sex differences were nonetheless similar in both species, emphasizing that there still exists a pronounced difference in shape between sexes independent of size. In contrast, latitudinal clines were independent of size, a pattern that was unexpected because both species show latitudinal patterns in wing size (Bergmann cline in *S. punctum*, and a converse cline in *S. fulgens*; fig. 1).

Conclusions

Geometric morphometric studies offer great opportunities to investigate the relationship between plasticity and evolved responses to common environmental drivers, potentially enabling us to predict how complex phenotypes react to changing environments. In contrast to studies on *Drosophila*, we here show that although the effect of temperature on wing shape is somewhat conserved across species, it is not consistently related to clinal genetic differentiation in shape. Hence, local adaptation and its relation to plasticity is idiosyncratic and possibly affected by multiple different factors, including the underlying population structure and levels of gene flow. In contrast to wing shape, we found consistent clines in wing loading. Relative body size rather than shape may thus be under consistent selection. This is a pattern found repeatedly in various intra- as well as interspecific studies and suggests a more common phenomenon, which however clearly requires further scrutiny.

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General conclusions and future directions

This dissertation demonstrates the need and merit of integrating microevolutionary, experimental and comparative approaches in understanding the evolution of life history (syndromes) in the broadest sense.

The **first chapter** assessed potential interrelations between several large-scale geographic patterns of phenotypic variation at the comparative level. While there is support for consistent variation in body size and relative wing size following climatic gradients, suggesting local adaptation, there is only limited evidence for interrelations and confounding effects among Bergman's, Allen's and Rapoport's rules. The chapter nevertheless demonstrates that studying several traits simultaneously at minimum permits better interpretation in case of multiple, potentially conflicting trends or hypotheses concerning the macroecology and life history evolution of insects.

The **second chapter** investigated quantitative population differentiation in body size, development time and fecundity along a latitudinal cline in *Sepsis fulgens*. Clines were largely flat, though despite the small size and short development time of this species, body size decreased slightly with latitude. Contrasting the currently available data for sepsids with those from drosophilids showed that geographic variation in life histories (in particular body size) do not correspond well between these two clades. This is despite the fact that both families typically dwell on ephemeral decaying organic matter, have very short development times, and similar body size. Such idiosyncrasy may well be common. It is troubling; however, as it demonstrates that our current understanding lacks generality and is not yet complete enough to enable adequate predictions.

The **third chapter** investigated whether the generally greater phenotypic body size plasticity of females observed in insects is due to selection on size or a result of selection associated with the female reproductive role. This is difficult to assess because females are the larger sex in most studied insects. Comparing closely related pairs of populations and species with directional variation in SSD suggests that the larger sex generally is more sensitive to environmental variation. That is, females are not generally more plastic, and their increased plasticity is likely driven by selection on size and not by constraints relating to their reproductive role (e.g. to the availability of limited nutrients). This further implies that strong sexual selection on male size not only reverses SSD, but appears to do so by amplifying the plastic response of males (i.e. a form of V_{GxE}). Hence, sexual dimorphism and sex-specific plasticity are not necessarily independent from each other, but one may arise from the other and vice versa.

The **fourth chapter** investigated the relationship between sexual dimorphism and condition dependence (which represents a form of phenotypic plasticity) within and across species in more detail. Body size-corrected sexual dimorphism of seven morphological traits correlated positively with sex-specific condition dependence in all eleven species studied. This relationship not only held within species, but also across species. That is, when males evolve larger trait values compared to females, they show a corresponding increase in condition dependence (and vice versa). Sexual dimorphism and condition dependence hence share developmental underpinnings. This only applies to traits under strong directional sexual selection but not to other, non-sexual morphological traits such as wings or thorax size. As a result, sexual dimorphism itself can be condition-dependent, and these two concepts may be biologically interrelated and difficult to distinguish.

The **fifth chapter** investigated the mechanistic, physiological basis of SSD and sex-specific plasticity in the yellow dung fly *Scathophaga stercoraria*. Sex-specific size plasticity was mediated by faster initial growth of males that later became reduced by greater male weight loss during the wandering stage. In some environments, this weight loss can be so large that even though larval peak weight of males is higher than that of females, this size difference is lost during subsequent development to result in monomorphic adult size. Critical weight, a major determinant of adult size marking the mass at which a larva initiates pupariation, also showed sex-specific plasticity. The association between size determination and sex-specific plasticity suggests that the former is a likely target of selection on adult size. In addition, comparing different measurements of growth rates highlighted the importance of detailed assessments of ontogenetic growth trajectories for the understanding of adaptive size variation.

The **last chapter** dealt with the relationship between genetic latitudinal population differentiation and thermal plasticity in a complex phenotype, the

insect wing. Comparing patterns in two closely related sepsids with similar ecologies, we found that although wing allometry, sexual dimorphism and thermal plasticity show similar patterns in both species, latitudinal differentiation only mirrored thermal plasticity in *Sepsis punctum* but not in *Sepsis fulgens*. Such discrepancies may be driven by variation in gene flow and demography; hence, our results support the notion that genetic wing shape differentiation may be complex and idiosyncratic, even among ecologically similar, closely related species. In contrast to shape, clinal variation in wing loading showed a consistent decrease towards north. This is congruent with patterns in drosophilids at the intra- as well as the interspecific scale. Potentially linked to dispersal in the cold, clinal variation in relative wing size could hence constitute a common pattern among small insects, a prediction that warrants further scrutiny.

Future directions

The study of sexual size dimorphism and body size plasticity has a long tradition in evolutionary ecology (Darwin 1872, Rensch 1950, Cope 1885, Peters 1986, Reiss 1991). While the ultimate processes are by now rather well understood, our understanding of the underlying proximate mechanisms remains unsatisfactory (Badyaev 2002, Stillwell et al. 2010, Flatt and Heyland 2011). This is mostly due to a limited understanding of the genetic architecture and the molecular genetic/developmental processes leading to different sizes in males and females.

Particularly in comparative studies (here chapter 1), sexual size dimorphism is often regarded as a rather static trait, even though the significance of plasticity and genotype-by-environment interactions has been widely appreciated in quantitative genetics for decades (Lynch and Walsh 1998). This thesis illustrates that the evolution of sexual dimorphism, both in overall size (chapter 3) and relative trait size (chapter 4), is not independent from sex-specific plasticity (chapter 6). That is, (sexual) selection on size may target the genetic/developmental basis underlying sex-specific developmental plasticity of a trait (sex-specific epistasis, c.f. Bonduriansky 2007a,b), thus establishing a common developmental basis. Chapter 5 illustrates this, as size determination systems themselves are phenotypically plastic and sexually dimorphic. The effects of endocrine cascades on dimorphism and plasticity therefore must be intertwined. As such, the role of sex-specific developmental plasticity and its genetic underpinnings needs to be studied in more detail.

The findings reported here also re-emphasize that insect growth is non-linear and complex (chapter 5, Esperk et al. 2007, Tammaru and Esperk 2007, Tammaru et al. 2010). This necessarily exacerbates studying the mechanisms mediating plasticity or sex differences in adult size. Although detailed assessments of larval growth are tedious and not applicable in all systems, studying variation in growth rates and development time using linear estimates may be too simplistic and can obscure rather than reveal patterns. Current evolutionary studies, particularly at the population level, may hence fail to embrace the nonlinearity and discontinuity of growth (although there are many notable exceptions: Shafiei et al. 2001, Meister et al. 2017, Vendl et al. 2018). On that note, there is also a clear need for better understanding size determination systems in the context of SSD and plasticity. Such mechanisms have been studied in detail in very few species (Davidowitz et al. 2003, Nijhout 2003, 1984, Nijhout and Davidowitz 2009, Mirth and Riddiford 2007). Given the variation found between these taxa, size determination may be key to uncover not only the mechanisms that lead to genetic and plastic responses, but also the underlying physiological constraints. Although this requires major scientific efforts, such data may be critical and represent a worthwhile additional route to understanding body size and SSD evolution.

In general, this work shows that, in contrast to restricting oneself to few linear measures, appreciating the multivariate nature of life-history syndromes (i.e. in the case of clinal variation), growth and morphology (e.g. the insect wing) should lead to a better understanding of the processes and mechanisms in action. Although far more demanding and challenging, such approaches enable better and more general conclusions.

To conclude, the evolution of life histories, body size and their plasticity continue to be subject to considerable scrutiny. Given the development of novel powerful molecular genetic tools (such as CRISPR/Cas, next generation sequencing, etc.), investigating the molecular basis even of complex phenotypes has become feasible. Repeating the tenor of multiple other modern evolutionary biologists (Flatt and Heyland 2011, and authors therein), our ultimate goal should now be to uncover the proximate, mechanistic drivers of life history evolution in order to better understand and predict it.

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Publications in peer-reviewed journals

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one species to the Alpine fauna while questioning the synonymy of Sepsis helvetica Munari, 1985. 8th International Congress of Dipterology in Potsdam, Germany.

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Submitted manuscripts currently under peer review

Rohner P.T. and Blanckenhorn W.U. (submitted to *The American Naturalist*) A comparative study of the role of sex-specific condition dependence in the evolution of sexually dimorphic traits.

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